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BUSINESS MATTERS OF THE INTERNATIONAL ACADEMY

FROM THE OFFICE OF THE PRESIDENT

In accordance with the Bylaws of the International Academy Article I, Section 3, an

INTERNATIONAL MEETING ON EXFOLIATIVE CYTOLOGY

is being planned for the year 1961, probably immediately preceeding the Third World Gynecology Congress. The place of the meeting will be Vienna, Austria.

Extracts from the Bylaws concerning the Scientific Session (*Article 1, Section 10*) : ". . . Papers presented at the Scientific Session shall be original papers which have never been presented or published. Material which has already been published or presented elsewhere may be considered in panel discussions. Fifty per cent of the Scientific Session shall be devoted to original papers, and fifty per cent of the time to panel discussions. . . ."

For details concerning the International Meeting write to Dr. Ruth M. Graham, Roswell Park Memorial Institute, 666 Elm Street, Buffalo 3, New York, U.S.A.

LETTERS TO THE EDITORS

HISTOCHEMICAL EVIDENCE OF VAGINAL CORNIFICATION

TO THE EDITORS:

We were greatly interested to see, in ACTA CYTOLOGICA Vol. II, No. 1, 1958, the Opinion Poll on Cytologic Definitions, particularly those parts which discussed the applicability of the terms "cornification" and "keratinization" to cells of the human vaginal epithelium. Opinion seemed to be intractably and unfortunately divided on the two questions (a) whether or not the epithelium does in fact cornify (or keratinize) and (b) which of the two terms properly describes the process if it takes place. We should like to mention some earlier work (1) which was an attempt to clarify the cornification status of this tissue by histochemical means.

Biopsy specimens were used, taken from the vaginal walls of 28 women in different stages of normal menstrual cycles. The following criteria were taken to be indicative of cornification in microscope preparations:

(a) Presence of protein-bound disulphide groups in cell walls and cytoplasm.
(b) Birefringence on examination with polarized light.
(c) Intense red staining with Papanicolaou's, and orange-yellow with Gurr's M. F. 4., vaginal smear stains adapted for use on sections.
(d) An intense and selective deposition of Heidenhain's hematoxylin in cell walls and cytoplasm.

With but one exception, where the Orange G staining was aberrant, the congruence between these techniques was complete, the reaction being specifically localized in a narrow zone of the epithelium. Taken together, we consider that these results reveal the existence of a histochemically definable zone of the epithelium in which cornification is undoubtedly taking place, with keratin being laid down in the cell walls and peripheral cytoplasm. We believe, however, that normally the process remains incomplete and that it is only in prolapse, leukoplakia, and abnormal hormonal conditions, etc., that the deposition of keratin comes to occlude the cell completely.

This zone of cornification appears in an intermediate position in the epithelium during the follicular phase of the cycle, thickening and approaching the surface at mid-cycle. Its cells are progressively desquamated into the vaginal lumen during the early luteal phase. Such a zone was originally described by Dierks (2) in 1927, but subsequent authors have denied or reinterpreted its existence on histological grounds. Dierks' contention is, however, justified by the rediscovery of the zone as a histochemically distinct feature of the healthy epithelium and cannot be dismissed as a mechanical artefact. The staining reactions which characterize it are identical with those shown by keratinized tissue from other sources, including human and mammalian epidermis, the vaginas of rats and mice at estrous, and the vaginas of estrogen-treated ovariectomized mice.

Moreover, the appearance and fate of this zone are in accordance with the familiar cyclical variation in numbers of cornified cells in vaginal smears and are clearly related to the hormonal changes which accompany the menstrual cycle. The value of vaginal smear stains in differentially staining cornified cells is, in general, confirmed.

Throughout the paper referred to (1), the term "cornification" was used. A review of the precedents established in the literature, however, showed no reason why "keratinization" should not equally well have been employed. We are not aware of any mutually exclusive definitions of these terms. However, agreement on definitions is obviously of prime importance to those using vaginal smears for clinical diagnosis, who might for instance prefer to use "cornified" and "keratinized" to denote cells which have reached different stages in the one metabolic process. Our point is simply that the process of cornification or keratinization does occur in the healthy human vaginal epithelium.

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1. Asscher, A.W., Turner, C.J. and de Boer, C.H.: J. Anat. Lond. 90: 547, 1956.
2. Dierks, K.: Arch. Gynaek. 130: 46, 1927.

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TO THE EDITORS:

Reference is made to the above Letter to the Editors by Drs. Turner and Asscher. According to my own histochemical studies on the vaginal epithelium, I fully agree with their statements that there is a histochemically appreciable zone of keratinization in the vaginal epithelium. In addition to the mentioned reactions I would like to point out the rather diffuse brownish staining of the cytoplasm of the superficial stratum, indicating the participation of phosphomonestrases in the synthesis of keratin. I also agree that the process of keratinization usually remains incomplete, i.e., that there is usually a higher content of sulfhydryl groups than of disulfide groups. The completed process of keratinization, however, becomes evident only under pathological conditions: (a) in patients with prolapse and/or leukoplakia one finds a histological pattern similar to the one of the epidermis, (b) in patients with carcinoma in situ one encounters layers of keratinized superficial spindle cells and (c) in patients with invasive cervical carcinoma one also sees horn pearls containing true keratin in addition to the pattern observed in carcinoma in situ. Contrary to normal, healthy vaginal epithelium, these pathologically altered epithelia do not respond to hormonal stimuli, i.e., because of their autonomous character they have ceased to respond to the differentiating stimuli of estrogens.

Doz. Dr. Hanns-Werner Boschann
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TO THE EDITORS:

During the Symposium on Applied Cytology in Brussels (Acta, Union Against Cancer 14: 403, 1958) we have discussed the studies of Asscher, Turner and de Boer and stated that we agreed with their histochemical findings. We do not believe, however, that one deals with a true keratin formation in the vaginal epithelium. According to our findings, there is occurrence of a diastase resistent polysaccharide which is histochemically definitely different from the simultaneously present glycogen. This is characteristic for a delayed keratin formation. The occurrence of the positive reaction appears at the same time as the occurrence of the eosinophilia. The same patterns are observed occasionally in the epidermis and always in distinct periods of the hair formation. The keratinization of the vaginal epithelium is arrested normally in this stage.

It seems to us, therefore, that one is justified in referring to the cells from the vaginal epithelium as "keratinized" or "cornified." At most, one deals here with a pre-keratin formation. The occurrence of eosinophilia has nothing to do with the process of cornification.

Dr. H. Ebner and Prof. P. Stoll
Universitaets-Frauenklinik
Heidelberg, Germany

This is a brief answer to the above letter sent to ACTA CYTOLOGICA by Drs. Turner and Asscher. I am in general agreement with their views with the exception of the sentence which reads as follows:

"We believe, however, that normally the process remains incomplete and that it is only in prolapse, leukoplakia and abnormal hormonal conditions, etc., that the deposition of keratin comes to occlude the cell completely."

As I already stated, I believe that the true keratinization process as seen in cases of prolapse or leukoplakia differs from that of cornification as seen in the superficial layers of the epithelium of the vagina mainly during the period of high estrogenic stimulation. True keratinization is generally associated with the presence of a stratum granulosum, which suggests a distinctive histochemical process comparable to that seen in the skin. The exfoliated cells also show differential morphological and histochemical characteristics. Cornified cells are, as a rule, nucleated and eosinophilic in contrast to the distinctly orangeophilic keratinized cells, in which the nucleus has almost entirely disappeared.

With regard to the terms "cornification" and "keratinization," as now used in exfoliative cytology, I feel that they should be retained as long as no substitute terms are suggested, that would be more appropriate for describing the above indicated morphological and histochemical differences.

George N. Papanicolaou, M.D., Ph.D.
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I was very pleased to read the above letter by Drs. Turner and Asscher, because it is a summary of an important contribution to the problem of human vaginal cornification or keratinization which every cytologist should read. It would have been very helpful if we could have discussed this paper during the previous symposium on cytological definitions. I thank the authors for sending me a reprint of their interesting publication which I hope will help us to bring out a clearer definition of vaginal cornification or keratinization.

During some research work done a few years ago on the histology of a normal human vagina, I also saw, with a modified Shorr technique, an eosinophilic epithelial zone in vaginal sections which corresponded to the Dierks layer, but it was covered by a "basophilic" superficial layer (biopsies were taken between the 8th and 12th day of the cycle). At that time I thought that this astonishing staining reaction was an artifact, and now I am glad to see that Turner and Asscher have demonstrated the same reaction with a better technique.

The authors conclude that, in general, there exists a good congruence between the eosinophilic reactions of the usual differential staining techniques and the specific reactions of the special techniques used for the demonstration of cornification or keratinization. I agree with these conclusions, but I think that they should not be accepted for every staining technique. It has been demonstrated by Papamiltiades that the eosinophilic or yellow staining reaction of the vaginal epithelial cells depends upon the composition of the staining mixture. For example, with the mixture of naphtol yellow and eosin, the superficial cells will stain yellow and the "basophilic" intermediate and parabasal cells will appear eosinophilic. With this technique eosin will give a paradoxical eosinophilic reaction in otherwise cyanophilic cells, a reaction which cannot be accepted as evidence of keratin.

If one considers the histochemistry as presented by the authors, we now have to settle the cytological definition. We have to choose between a scientifically correct definition or a definition of personal convenience which is scientifically incorrect:

DEFINITION I: Histochemistry and international language do not permit the usage of the terms cornification and keratinization as different terms, since they are scientifically synonymous. Therefore, if one prefers to use these terms in a scientific manner, one can accept only the following definition: Cornified or keratinized cells are cells which contain keratin in various amounts. As these cells with the usual differential staining techniques, in general, have a red or eosinophilic reaction, the terms eosinophilic, keratinized and cornified cells would be synonymous.

DEFINITION II: For practical purposes one could accept the term keratinized cell only for such cells showing a complete keratinization, with loss of the cell nucleus. I think that now there exists a general agreement on this practical definition, as shown in the previous symposium on terminology. Under this definition cornified cells would be the other cells, showing a definite content of keratin, but in which the keratinizing process has not reached its maximum degree. Keratinization and cornification would be qualitatively synonymous, and the difference would be only quantitative. But, as the previous symposium has shown, there exists no clear and generally accepted definition for "cornified cells," a term which means different things for different cytologists. If we would like to remain upon a scientific basis, we can accept as "cornified" only those cells which show an eosinophilic staining reaction of the cytoplasm, and the presence of their nuclei would distinguish them from the keratinized cells, which are without nuclei. But the application of the term "cornified cells" to cyanophilic or "basophilic" cells, even if they present some features of superficial cells, is scientifically not defensible.

From the interesting work of Turner and Asscher it appears that definition No. I would be the best. Maybe, for practical purposes, one could maintain the term keratinized cell for squames (cells showing a complete keratinization). There exists no scientific reason for permitting different usages for the terms keratinization and cornification, which are synonymous in the international scientific language. It seems preferable, in order to avoid misunderstandings, to abandon completely the term "cornified cells." If this term has to be maintained, it should be applied only to nucleated eosinophilic cells, but never to other cells.

Professor J. Paul Pundel
23, Ruf Albert 1er
Luxembourg, Luxembourg

In Volume II, Number 1 of ACTA CYTOLOGICA, devoted to terminology, there was fair agreement that the normal vaginal epithelium did not contain true keratin. Several contributors to that symposium stated that there may be keratin-hyalin granules or in some instances pre-keratin, but that true keratin, as demonstrated by the presence of protein bound disulphide groups, was absent. In the Turner's and Asscher's communication to the editor they state definitely that the presence of protein bound

disulphide groups can be demonstrated in the normal vaginal epithelium and emphasize this by their last sentence, "Our point is simply that the process of cornification or keratinization does occur in the healthy vaginal epithelium."

I regret that Turner and Asscher did not describe their method for the histochemical determination of keratin. When such divergent views as these are present, one immediately becomes concerned with the techniques of preparation. By this I do not mean to imply that the author's technique may be in error. For all I know it may be superior to other techniques. However, unless the same techniques are used in exactly the same way by different investigators it is often difficult to compare the results.

This communication seems to me to raise a serious point. The premise for discarding the terms cornification and keratinization was based on the fact that many investigators stated that the superficial cells of the vaginal mucosa did not contain keratin. This is a problem that should lend itself easily to solution.

First, detailed accounts of the techniques for the demonstration of keratin should be communicated to the editor. It would be preferable to have, not only the details of the procedure, but any pitfalls that might be encountered, plus any difficulties in interpretation. After these staining procedures had been published, one could try them on a series of cases and send the results with photomicrographs to the editor. If ten investigators were willing to enter into such a program and investigate twenty patients, not only could this question of whether or not true keratin is present be resolved, but the reproducibility of particular histochemical methods could be determined.

It is my impression that this should be one of the purposes of ACTA CYTOLOGICA, to solve quickly and definitely exactly such questions as raised by this letter. I will be perfectly willing to do such a study in my own laboratory. Will others join me?

Ruth M. Graham, Sc.D.
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Buffalo 3, New York, U.S.A.

CYTOLOGISTS INTERESTED IN PARTICIPATING IN THE ABOVE TEAM WORK SUGGESTED BY DR. GRAHAM ARE INVITED TO CONTACT THE EDITORIAL OFFICE. Ed.

THE OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN CARCINOMA IN SITU

TO THE EDITORS:

Reference is made to the papers on the above subject in Vol. II, No. 2. According to our experience, spindle-shaped squamoid cells are very rare in smears of histologically-proven carcinoma in situ. We re-checked part of our material and again out of a total of 38 cases of carcinoma in situ (Oberflächencarcinom), we found only three smears with spindle-shaped squamoid cells. We have too little material of the early invasive type of lesion to permit definite statements. In four of the latter cases we could not find any spindle-shaped squamoid cells.

Professor Hans-Klaus Zinser
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INFLUENCE OF SPERM ON THE CYTOHORMONAL PATTERNS

TO THE EDITORS:

May I put the following inquiry to the readers of ACTA CYTOLOGICA and ask them whether or not there is any experience with the following topic:

I have been asked by a colleague whether or not the minimal dosages of male sex hormone which are found in the sperm would exert a stimulus on the vaginal epithelium. The question is also, whether or not the normally estrogen stimulated epithelium would depict such stimuli at all. Secondly, I would also be interested in knowing if any experience with patients with atrophic epithelium has been done. I would be very grateful to receive any references in the literature and also the name of any individual who has worked in this particular area.

Dr. Wolfgang Korte
Universitaets-Frauenklinik
Bonn, Germany

SPECIAL PANEL FOR INTERESTING SPECIMENS

TO THE EDITORS:

May I suggest that an exchange of opinion on especially interesting and/or smears with special problems be inaugurated within the framework of the International Academy. I believe that this could also be of special advantage and interest in those selected cases which are not necessarily reported on in an individual publication. The organization of this endeavor possibly could be set up quite similar to that of the Symposia by Correspondence. However, the time period for discussion among the evaluators of these special specimens should be cut to four to six weeks. This could probably be achieved by limitation of the groups of evaluators to a small panel, or by the formation of individual teams of evaluators who are specialists in various fields. The participants on these panels would agree that their opinion be used in publications, either with or without mentioning their names.

Professor Bickenbach and I would be very glad to send some specimens from time to time for such evaluations, as well as to participate in the discussion of submitted slides.

Dr. Hans-Jürgen Soost
Maistrasse 11
München 15, Germany

THE READERS OF ACTA CYTOLOGICA ARE INVITED TO EXPRESS THEIR OPINION AS TO THE DESIRABILITY OF THIS SERVICE SUGGESTED BY DR. SOOST, AND FURTHER COMMENTS ON THE TYPE OF SERVICE RECOMMENDED WOULD BE APPRECIATED. Ed.

DEFINITION OF DYSKARYOSIS

TO THE EDITORS:

In regard to the three photomicrographs and questions submitted by Dr. del Sol (Vol. II, No. 1, 1958, pages 12 - 14), I would reply as follows:

1. Do the cells in photomicrograph Figure 1 exhibit parabasal cell dyskaryosis or are they differentiated cancer cells? All the cells present in the field are differentiated cancer cells, and I would consider them all third type differentiated squamous cancer cells. Though some of the cells have more cytoplasm than others, none of them have adequate cytoplasm, i.e., the distance from the nuclear border to the cytoplasmic border is not greater than the maximum diameter. For instance, consider the cell at the bottom of the field just to the left of center. The maximum diameter of the nucleus is 1.0 cm. at this magnification, but the distance from the cell border to nuclear border is only 0.5 cm. For practical routine work we never actually measure these distances but merely judge them visually. It is certainly true that there is a spectrum of cells from the true differentiated cancer cell to the obvious dyskaryotic cell, and occasionally they may be present in the same field. However, in this photomicrograph the cancer cells are remarkably similar.

Though the cellular borders in Figure 2 are somewhat difficult to see, an occasional cell is distinct enough to classify as a third type differentiated squamous cancer cell.

2. In answer to this question, I believe one should describe the cluster of cells as accurately as possible and not merely on the basis of the predominant cell type. If Figure 1 did show dyskaryotic cells (which it does not) one could describe it as a field containing differentiated squamous cancer cells and dyskaryotic basal cells.

3. I would consider the large cell in Figure 2 an intermediate dyskaryotic cell, not superficial.

4. It is my belief that we should relate the dyskaryotic cells to their normal counterpart. Basal dyskaryosis occurs in cells which are of the size of basal cells and whose shape is oval or round. Intermediate dyskaryosis occurs in cells which are the size of or sometimes larger than the normal intermediate. The nucleus is well preserved and the chromatin irregularity is easily recognized. By definition the normal superficial cell has a pyknotic nucleus and no chromatin particles are observed. I feel this same criterion should apply to the superficial dyskaryotic cell. If a cell the size and shape of a superficial cell has a large irregular pyknotic nucleus, it can be classified as a superficial dyskaryotic cell. I do not believe it is of any consequence if either intermediate or superficial dyskaryotic cells are larger than their benign counterparts. It is the shape of the cell and the sharp corners which allow us to identify them.

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ADVANTAGES AND DISADVANTAGES OF THE MAY-GRÜNWALD-GIEMSA STAINING TECHNIQUE FOR EXFOLIATIVE CYTOLOGY

TO THE EDITORS:

Reference is made to the papers by Dr. Luksch of Prague and Dr. Lopes-Cardozo of Leyden on the Advantages and Disadvantages of May-Grünwald-Giemsa Technique in ACTA CYTOLOGICA, Volume 2, No. 2, 1958.

As far as the diagnosis of cancer is concerned, I agree with Luksch. My own experience, based mainly on cytology in internal medicine, supports his viewpoints. Furthermore, there is no doubt that the Giemsa procedure is less time consuming and wastes less material than the Papanicolaou procedure. However, there is a disadvantage which seems of some importance: It is the remarkable lack of transparency of uncovered dry smears. Specimens with a high content of mucous will demonstrate this disadvantage in a striking way. Although the transparency of dry specimens prepared according to the Giemsa technique can be improved remarkably by wetting and covering them with a cover glass, there is still another disadvantage of applying the Giemsa technique in gynecological cytology. This is the uselessness of the Giemsa technique in the hormonal evaluation of the patient, as outlined by Luksch. What is it that makes cytodiagnosis in gynecology so efficient? It seems to me to be the possibility of answering three important questions by doing only one procedure, namely: (1) Is there evidence of cancer, (2) is there evidence of inflammation and (3) what is the hormonal status? The Giemsa technique, which does not permit the answer of these three questions, therefore, does not fulfill the requirements in gynecological cytology.

The good results obtained by using the Giemsa stain, which Lopes-Cardozo described can be endorsed by my own experience in many ways. Mainly, this applies for the thin-needle-puncture cytology performed in internal medicine. This procedure often permits the preparation of very thin and homogenous smears. This is important in that in thin smears, the artifacts due to shrinking by air drying are very small and the stain becomes very homogenous. Furthermore, in thin preparations the problem of transparency does not arise as it does in thick smears especially when the specimen is rich in mucous. Although transparency can be improved in such cases by wetting the smears, I prefer the Papanicolaou stain in those cases, if the problem is not a hematological one.

However, in internal medicine we must always consider the question of a hematological disease or an involvement of the lymphatic system. Thus, we have to reserve one or two unstained smears for a possible Giemsa stain. This is also true in such cases where the Papanicolaou stain is better. It seems to be necessary because the differentiation of the cytoplasm is insufficient in the Papanicolaou stain. In generalized diseases of the hematopoietic and lymphatic systems, even an insufficient Giemsa or Papenheim stain might be more informative than the brilliant Papanicolaou stain, as Lopes-Cardozo also points out.

In our laboratory, if there are no hematological problems involved, the Papanicolaou stain is used as a routine procedure for mucous-rich material or thick smears. Mainly, this is done because we believe that it is easier for medical technicians to prepare a good Papanicolaou stain than a good Giemsa stain. Perhaps this is due to the many similarities found in histological staining procedures and to a lowered pH sensitivity of the Papanicolaou stain. Furthermore, cancer screening is more easily done when using the Papanicolaou stain, not by adding qualitative cancer characteristics, but by not staining the details which are not important and not diagnostic for cancer. This disadvantage is even more pronounced in the phase contrast technique than in the Giemsa stain. Although keratinization is detectable also by using the Giemsa stain, it is more pronounced and obvious with the Papanicolaou stain. As squamous cell carcinoma occurs very often, this advantage of the Papanicolaou stain should be considered.

The sensitive reaction of the Giemsa stain to pH alterations or para- and dysproteins which are sometimes diagnostic in hematology, seems to only be a disadvantage in cytology when examining secretions or effusions.

This is due to the fact that the alteration of the pH or the occurrence of atypical proteins in cytology is not necessarily caused by the basic disease, but is more often due to the various autolytic changes which take place.

Dr. Lopes-Cardozo points out that the ether-alcohol wet-fixation induces stronger shrinkage of the cells than the Giemsa stain. Referring to my own experience, I am not able to confirm this finding when using both the Giemsa stain and the Papanicolaou stain with equally thin smears from the same specimen.

Possibly Dr. Lopes-Cardozo has compared thin needle smears which are Giemsa stained with thicker smears from other sources.

In the thin smears, the cells are adherent to the glass slide on a wide base. This means they are suffering a nonphysiological deformation which is, however, not disadvantageous for diagnostic purposes. Furthermore it prevents too great shrinkage during the fixation procedure. In the thick smears, however, using the wet alcohol - ether fixation and the Papanicolaou stain, the cells will be surrounded by mucous and will have no attaching point on the glass slide. So cells and mucous can shrink simultaneously during the fixation procedure. However, the artifacts of this shrinkage in thick smears during wet fixation procedure (this applies also to the methyl-alcohol fixation and the Dudgeon-Patrick wet-firm technique) are not as bad as in equally thick air-dried smears, where cells really become torn.

Therefore, it seems apparent that the minor shrinkage of cells in thin Giemsa stains has nothing to do with the fixation or staining procedure itself, but is only due to the adherence to the glass slide. Thus, the advantages of the Giemsa technique can be demonstrated in cytology only in thin smears.

Summarizing, I am able to confirm the good results of Lopes-Cardozo with the Giemsa stain. However, I should like to recommend a more differentiating procedure in non-hematological cases dealing with thick mucous-containing specimens.

Doz. Dr. Heinz Grunze
Stoelpchenweg 7
Berlin-Wannsee, Germany

TO THE EDITORS:

The above comments of Dr. Grunze are thoughtful and I agree with many of his statements. The stains on a hematoxylin basis have proven to be quite useful and perhaps permit a quicker screening for many people. Therefore, I can understand that many prefer the hematoxylin type of stain for exfoliative cytology - keeping an unstained slide in reserve for the Giemsa stain. However, I do not like to use it for dab- and touch-smears, abrasive cytology, needle-aspiration cytology and hematocytology. Perhaps this procedure might occasionally be useful in gynaecocytology also.

Dr. Paul Lopes-Cardozo
Nieuwe Plantage 23
Delft, The Netherlands

The May-Grünwald-Giemsa technique has been examined in the Laboratory of Exfoliative Cytology of the University of Chicago Clinics on cytological smears prepared from the female reproductive tract. The smears were compared with specimens of the same patient processed according to the routine Papanicolaou technique. It has been found that the May-Grünwald-Giemsa procedure is definitely inferior and cannot be recommended for use on cytological smears of secretions from the female reproductive tract. G. L. W.

CRYSTALS IN SMEARS

TO THE EDITORS:

It seems that the "Crystals in Smears" shown on the photomicrograph in Vol. II, No. 1, 1958, page 24, are granules of starch.

These appear in smears as circular or oval, disc-like plates of variable size and when stained by Papanicolaou's method appear softly rose-colored. Typical of starch granules, they can be seen clearly (due to light refraction) on playing with the micrometer screw. Starch granules get into the vagina in two ways:

1. when the patient uses cosmetic powder containing a starchy base and
2. when the examining physician uses gloves treated with a starchy powder.

May I point out another foreign body appearing in vaginal smears. When a smear is taken after the gynecological examination, it may happen that one sees softly translucent, whitish or yellowish crystals often by themselves, but sometimes in dense clusters; these have been found to be talcum granules.

Dr. Hans-Jürgen Soost
Maistrasse 11
München 15, Bavaria, Germany

TO THE EDITORS:

We have often observed crystals similar to those photographed on page 24 of Volume II, No. 1 of ACTA CYTOLOGICA, and can state quite definitely that they are talcum powder crystals (after they have gone through the fixation and stain).

We suspected powder from the gloves was contaminating the smears. To prove it to our satisfaction a powdered glove was shaken over a marked slide and using a second clean slide, the material was smeared on both slides from the same pipette. The smears first contaminated with powder from the

gloves, contained numerous crystals identical to those in the photograph. The smear on the clean slide showed no crystals.

Elizabeth H. Gray
(Cytology Laboratory)
Pathology, Ottawa Civic Hospital
Carling Avenue
Ottawa 3, Ontario, Canada

TO THE EDITORS:

The photograph published in ACTA CYTOLOGICA, Volume 2, Number 1, 1958, page 24, submitted by Dr. June Scudamore, represents starch grains of corn (Zea mays) rather than "crystals." We have observed these grains in cervical smears and similar ones when the powder from a sterile glove in the clinic was sprinkled on a clean slide. The grains stained black (starch) with Lugol's iodine solution; they remained on the slide in passage through the Papanicolaou staining process taking a faint orange stain.

Corn starch grains are shown in Wall's "Handbook of Pharmacognosy," 4th edition, 1917, figure 453, page 516. They are described as "polyhedric granules.... uniform in size with large, well-marked central hilum but indistinct layers."

Gardner M. Riley, Ph. D.
and
Eileen W. Macfarlane, Ph. D.
Department of Obstetrics and Gynecology
The University of Michigan
Ann Arbor, Michigan, U. S. A.

THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

INTRODUCTORY REMARKS

The Symposia of ACTA·CYTOLOGICA are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

The final detailed program will be published in ACTA CYTOLOGICA immediately preceding the one where these topics are to be considered, under the heading, THE NEXT SYMPOSIUM.

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers.* These principal papers will then be considered by persons identified as *Discussants.* As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 5 × 7 inches (i.e., 12 × 18 cm) and should show a proportional 10μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers.* Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the *end* of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

Reprints: Authors may receive reprints of their papers by ordering these reprints before the particular issue goes to press. There will be a nominal charge for reprints: $6.00 per page for the first one hundred copies, and $3.00 per page for each additional hundred.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les symposia: En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

Le bureau de rédaction établira le programme définitif et détaillé des discussions qui sera publié dans les ACTA CYTOLOGICA précédant immédiatement le symposium, sous la rubrique PROCHAIN SYMPOSIUM.

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 12 × 18 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure aît l'impression d'assister à une discution réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à *la fin* du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.*

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limites comprenaet:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

Tirés-à-part: les auteurs pourront obtenir des tirés-à-part de leurs communications en les demandant avant la mise sous presse des ACTA CYTOLOGICA publiant leurs articles. Les tirés-à-part seront facturés: $6.00 par page de texte pour le premier cent et $3.00 pour chaque centaine supplémentaire.

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

Die Schriftleitung bereitet das Programm mit allen Einzelpunkten vor, und veröffentlicht dieses Programm in dem Heft, das dem betreffenden Symposion vorausgeht, unter dem Titel DAS NÄCHSTE SYMPOSION.

Instruktionen fur Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 12 × 18 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Die Bibliographie der Referate und der Diskussions-Vortäge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

Termine für Beiträge: Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

Sonderdrucke: Autoren können Sonderdrucke ihrer Beiträge bestellen, bevor die betreffende Ausgabe in Druck geht. Die Schriftleitung muss diese Sonderdrucke berechnen und wird einen Betrag von $6.00 pro Seite und 100 Sonderdrucke, und einen Betrag von $3.00 für jedes weitere Hundert erheben müssen.

SIMPOSIUM ESCRITO DE ACTA CYTOLOGICA

El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

Sistema de selección de materias para el simposium: Con sugestiones recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en ACTA CYTOLOGICA con dos numeros de anticipación a la fecha de su posible publicación, bajo el epígrafe de "SIMPOSIUM FUTUROS."

La Oficina Editorial confeccionará y publicará una lista detallada del programa de la discusión en el número de ACTA CYTOLOGICA immediatamente anterior a aquel en que han de ser incluidos los temas, bajo el epígrafe de: EL PROXIMO SIMPOSIUM.

Participación en el Simposium Escrito: No habrá restricción alguna sobre el número de puntos de discusión en los que cualquier autor desee participar.

Instrucciones a los Autores: Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 600 palabras para los trabajos principales y 200 palabras para las contribuciones de los comunicantes. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusion. Las microfotografías y tablas deberán enviarse en forma de copias fotográficas amplias. A ser posible de 5 × 7 pulgadas (12 × 18 cms). *Cada figura deberá acompañarse de su correspondiente leyenda.*

Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales.* Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas.* Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda.

Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión asi como a otras directamente relacionadas con el tema.

La bibliografia, tanto de las ponencias como de las comunicaciones deberá redactarse de la misma forma que figura en el American Journal of Obstetrics and Gynecology, *al final* del trabajo. *Toda opinión o publicación citada deberá tener su correspondiente referencia en la bibliografía.*

Fechas para las contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

1°. Fecha límite para acuerdo de contribución,
2°. Fecha límite para las ponencias,
3°. Fecha límite para las discusiones,
4°. Fecha límite para las anotaciones finales.

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Carlo Mossetti, Torino, Italy
Constantin Herovici, Villejuif, Seine, France
Guillermo Terzano, Buenos Aires, Argentina
Erica Wachtel, London, England, U. K.

Symposium A

ENDOMETRIAL CANCER CYTOLOGY

HISTOMORPHOLOGY OF ENDOMETRIAL CARCINOMA

JEAN A. de BRUX
Paris, France

Endometrial carcinoma arouses but little interest on the part of clinicians, once the pathologist's diagnosis has been made. In fact, these tumors have a reputation of relative benignity, at least as long as the tumor has not perforated the serosa.

Yet the endometrial adenocarcinoma is of interest because of the important problems it raises: (1) the transformation of an active, normal but benign hyperplasia into a carcinoma, (2) the fact that it presents structural anomalies deriving from its organogenesis, and (3) that its prognosis is not always as benign as is generally thought.

A. The Classic and Indisputable Patterns of Adenocarcinoma

Two aspects are characteristic and are found either separately or jointly:

1. Pseudo-glandular type, with more or less marked differentiation.

2. Papillary form, with long, thin connective-vascular axis with abnormal pluristratified cylindrical cells with tendency towards organization into pseudo-glands.

Novak classifies the carcinomas into grades, based on the perturbations of the glanduliform architecture and especially based on the number and degree of the cellular anomalies:

Grade I: malignant adenoma
Grades II, III, IV: in which the number and degree of the architectural and cellular anomalies are increasing.

This classification fits the majority of the cases; however, we have seen some cases perfectly differentiated, formed of small glandular formations showing anomalies and without myometrial invasion, but which, less than 6 months after surgery, recurred in the vaginal scar, as well as in the pelvis.

B. Other Atypical Forms of Uterine Adenocarcinoma:

1. Adenoacanthoma - For the cytologist this form poses the difficult problem of determining whether an adenocarcinoma or an epidermoid carcinoma is involved.

2. Clear-cell carcinoma - These are found in the ovary, uterine corpus and endocervix, and their mesonephric origin is almost certain (Figs. 1, 2).

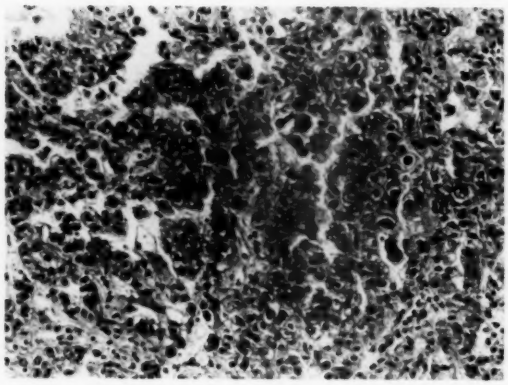

Fig. 1

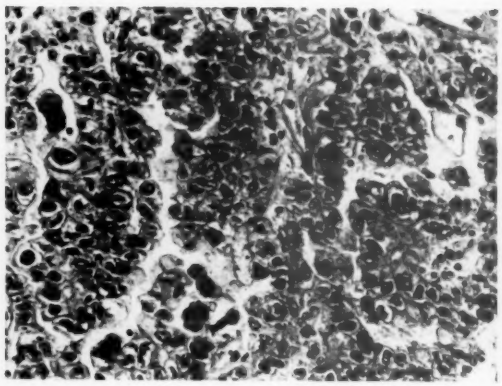

Fig. 2

However, these forms are rare, and one should be careful not to confuse them with certain endometrial atypias (1,2,3) which are related to the persistence of peri-ovular vestiges (decidual or chorionic villi) (Figs. 3, 4).

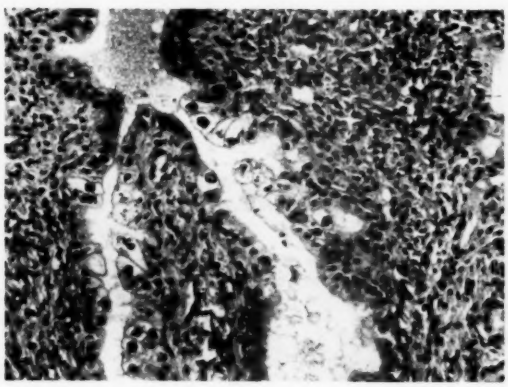

Fig. 3

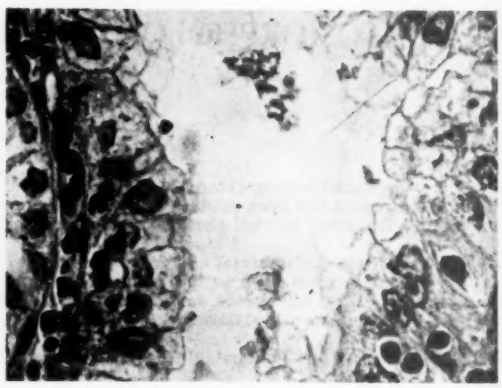

Fig. 4

3. Another form, more rarely seen, is the uterine carcinosarcoma, in which adenocarcinoma and sarcoma of the "cytogenous chorion" (Fig. 5) are found simultaneously.

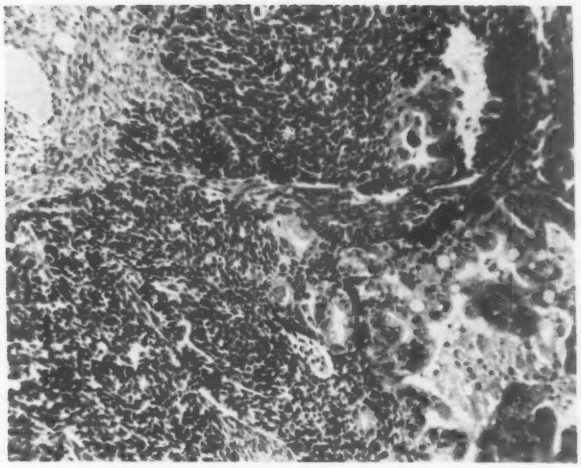

Fig. 5

C. There remains the controversial question of the adenocarcinoma in situ (4), in the interpretation of which various authors are not in full agreement. Its general aspect is that of hyperplasia. The glandular architecture is retained, without the anomalies of adenocarcinoma. Glands positioned back-to-back are found in the interstitial stroma. The cytoplasm is pale, sometimes eosinophilic; the nuclei are irregular, sometimes voluminous and crowded. According to Hertig (5), the term is synonymous with "carcinoid hyperplasia," "Novak's hyperplasia," or "adenomatous hyperplasia." I believe that the term carcinoma in situ should be perserved and that this diagnosis should only be made with caution. It is not unusual to find a very active adenomatous hyperplasia showing numerous and sometimes abnormal mitoses, but which nevertheless does not permit the diagnosis of cancer. In general, all hyperplasias undergoing hormonal stimulations are relatively non-suspicious for malignancy, notwithstanding abnormal patterns that may be present. The very pale or eosinophilic cytoplasm is not specific for malignancy but indicates cytochemical disturbance, frequent in cellular dystrophies (transformations of the mammary lobules, oncocytes of the salivary or thyroid glands, etc.). A feature of importance, in my opinion, is the architectural character described by Hertig: the glands back-to-back, with a slender stromal axis, anomalies and frequent mitoses. But in one case where all these characteristics appeared simultaneously on the histological specimen and which was diagnosed as carcinoma in situ, the vaginal smear showed - and with good reason - such abundance of abnormal cells as to lead us to insist on the immediate surgical exploration of the patient. To our great surprise, the exploratory laparatomy showed the presence of adenocarcinoma that had already penetrated the uterine serosa. In view of the above statements, I believe that the diagnosis of endometrial carcinoma in situ, which is a very difficult one, should be reserved for those relatively rare cases in which there is a strong possibility of cancer. Until a definite criterion of malignancy has been found, it is merely by the sequence of events in the follow-up of the case that we are able to distinguish the "true" from the "false" endometrial carcinoma in situ. Personally, I have seen only a few adenocarcinomas before the menopause. In our own collection which amounts to 250 cases of adenocarcinoma, we found only 3 cases before menopause. The youngest patient was 42.

Corscaden, Fertig and Gusberg (6) reported 318 patients with hyperplasias, of whom five developed carcinoma - a 1.5% chance of developing cancer, in women with endometrial hyperplasia.

In my opinion, however, attention should be directed not so much towards the number and activity of the mitoses and the cellular anomalies, but rather towards the general morphology - size and amount of chromatin and contents of nucleic acids, as Novak (7) has stated. In fact, pathologists find the close relationship among the different tumors of the genital tract striking. Certain glandular and papillary tumors of the ovary resemble some uterine adenocarcinomas so closely that they may be misinterpreted, whereas certain varieties of uterine carcinoma resemble mesotheliomas (Fig. 6). On the other hand, I have seen a peritoneal mesothelioma (confirmed by Dr. Fred Stewart), the aspect of which was that of an uterine carcinosarcoma.

For somebody familiar with uterine embryology and the characteristics of different tumors, it would seem beyond doubt that the "malignancy potential" of a tumor should be sought in the morphology and the cytochemistry of the cellular elements. The more they resemble the embryological elements from which they are oncologically derived, the greater will be their degree of malignancy. Thus, the most rapid penetration and extension is seen in those varieties of tumors the cells of which remind one of mesothelial cells with their different papillary, trabecular, and even pseudo-canaliculary patterns.

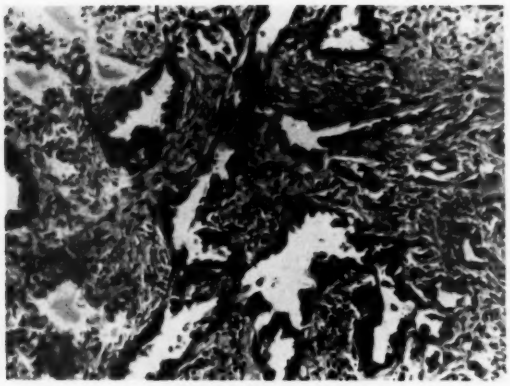

Fig. 6

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WOLFGANG KORTE

Bonn, Germany

Every classification and description of carcinoma of the uterine corpus necessarily demands opposition or requires completion. A formal, merely histological, classification seems to be suitable as a survey and introduction to a discussion on cytology of corporal carcinoma. In order to avoid confusion this survey must be concerned with the typical, definite forms, irrespective of the nomenclature of the frequently described (and controversal) tumors.

I. Primary Carcinoma of the Uterine Corpus

A. Adenocarcinoma or gland-like (gland-forming) cylindrical cell carcinomas: The macroscopic growth type is simple or multiple, tuberous, also broadly based fungoid, villous, diffuse or flat. Slow growth is usually observed. Consecutive muscular hypertrophy of the walls is present.

1. Alveolar carcinoma

The pure form is rare. Forms of the carcinoma adenomatosum with short glandular tubes belong to this type. Transitions to tubular forms nearly always exist. Usually the epithelial lining is monocellular, cuboid to flat-cylindrical. Regular structure of closely sited glands is seen. The single gland is often atypically formed. Nuclei are large, round to oval, and stain darkly. Destructive growth can always be found upon careful examination, even in the presence of highly differentiated patterns. Soft stroma is found. The tunica propria may be absent and mucous production is occasionally present.

II.

2. Tubular carcinoma

The tubular carcinoma corresponds best to its literal terminology and may imitate the structure of the endometrial functionalis. The tumor spreads first vertically into the myometrium, later horizontally. The glands appear slender,

long or short, sinuous, with cystically expanded tubes. Transitions to alveolar structure are encountered. The parenchyma of the well differentiated tumor grows in a single layer or is stratified and may be flat or highly cylindrical. Nuclei are clumsy, thick and rich in chromatin. Numerous mitoses are present which by no means must have atypical forms. Less differentiated cases show considerable nuclear and cellular polymorphism in addition to epithelial stratification. Sometimes production of secretions and transition into solid areas is observed. The stroma may be rich in cells and fibers. Relatively frequently, the predominant tubular type can be encountered in elderly women, very rarely in children. True mucus-producing carcinomas are rare. Carcinomatous corporal polyps belong in this classification.

3. Papillary carcinoma

Papillary carcinoma exhibits a mixed type with alveolar and tubular glandular lumina, surrounded by numerous little gracile and often large clumsy villi. The epithelial lining varies in thickness, is rarely monocellular, often stratified, cuboid, polygonal and highly cylindrical. Polymorphism of cell and nucleus is present. Tendency towards degeneration is found. Scarcely any secretion but cellular debris is seen in the gland-lumina. Strongly developed stroma often is present.

4. Papillomatous carcinoma

Papillomatous carcinoma is a rare tumor which represents, in the pure form, probably early stages of other types. Irregular formation of long papillae is often found. Rather regular, monocellular or bicellular lining of almost cylindrical epithelia is present. The less differentiated type shows clumsy, short papillae often with large, more polygonal cells and faintly staining nuclei. Destruction can always be seen. The stroma is rich in cells.

B. Solid Carcinoma

Solid carcinomas are less frequently found than the above tumors. The tissue of origin is nearly always demonstrable upon careful examination.

1. Carcinoma solidum deriving from the cylindrical epithelium (cancer simplex, scirrhosus et medullaris)

Carcinoma solidum is a tumor with coarse solid cones; consisting of medium-sized cells. The epithelia are polygonal, round, cuboid, frequently with large, nuclei. There is polymorphism of cell and nucleus, abundant mitoses, and tendency towards central necroses. There is also superficial ulceration with formation of little "sequestra." In scirrhous cancer, little round epithelia are found consisting almost only of nuclei, resembling cells of a round-cell sarcoma. The stroma is densely cellular or rich in fibers with tendency towards hyalinization.

2. Squamous cell carcinoma

True squamous cell carcinomas originating from the uterine corpus are rare. The structure does not differ from that of the cervical carcinoma. Keratinization is possible.

3. The so-called adenocancroid

The adenocancroid is primarily not a solid carcinoma. However, its essential characteristics are given by the keratinizing squamous epithelium. It must be classified in this group, therefore, when dealing with pronounced cases. Some gland-forming carcinomas also show the tendency to form islands of squamous epithelium (groups and nodules with and without horn pearls). These cases are not referred to here, for these are only special patterns, not typical pictures. The so-called adenocancroid forms the following patterns in nearly equal proportions: More or less undifferentiated glandular epithelium, solid epithelial parts and whole districts of keratinizing atypical squamous epithelium. The stroma is more or less moderately dense, often rich in cells. The tissue and cell formations are compatible with the above descriptions. Their arrangement is arbitrary. Squamous epithelial cells seem to originate directly from glandular tissue.

II. Secondary Carcinoma of the Uterine Corpus

A. Metastatic carcinoma

Metastases of ovarian carcinoma represent the majority of cases. It would require a separate chapter to describe their structure. Especially the mucinous carcinoma, metastases of metastatic ovarian tumors (Krukenberg tumor) and the so-called psammo carcinoma of the ovary may be mentioned here.

Fig. 1. So-called adenocancroid of the uterine corpus

Fig. 2. Adenocarcinoma of the uterine corpus: multiple cauliflower-like tumors

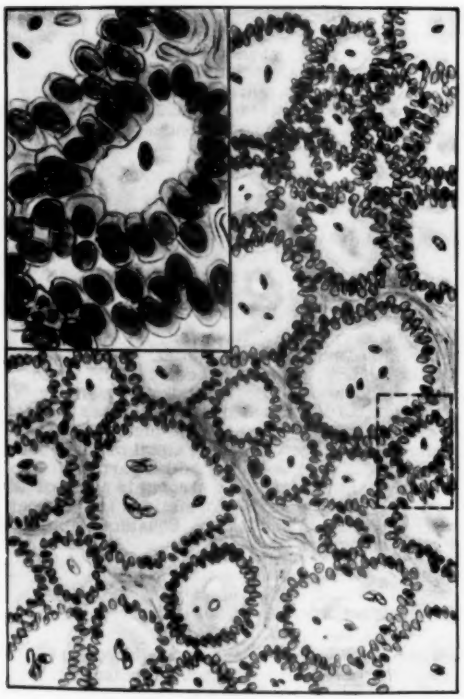

Fig. 3. Alveolar carcinoma

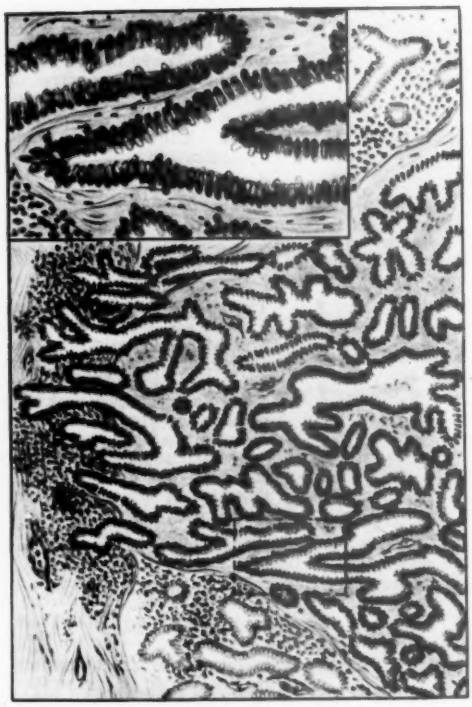

Fig. 4. Tubular carcinoma

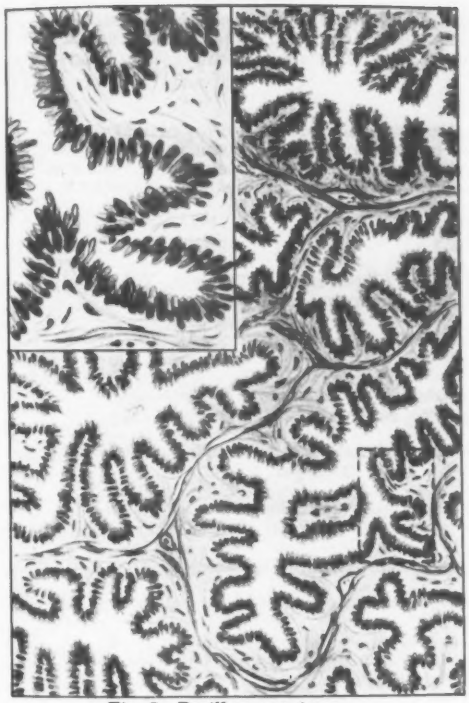

Fig. 5. Papillary carcinoma

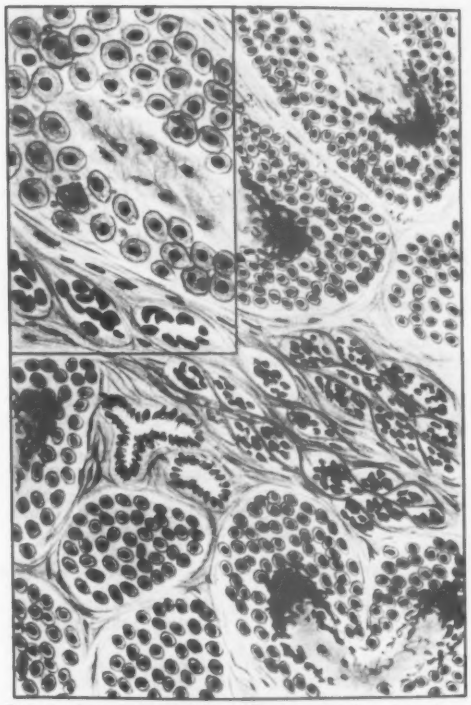

Fig. 6. Carcinoma solidum (Cancer simplex, scirrhosus et medullaris)

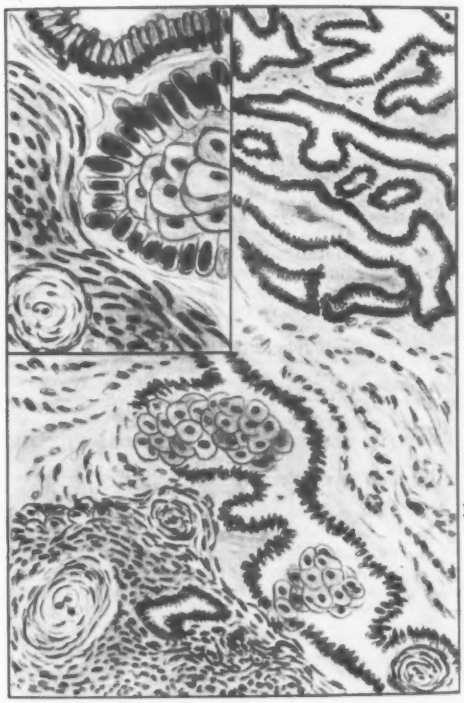

Fig. 7. So-called adencancroid

*All figures are schematized and drawn from macroscopic and microscopic original preparations.

Lymphatic metastases of tubal carcinoma are rare. Metastatic carcinomas are described of primary mammary tumors, bronchial carcinoma, of carcinomas of the stomach, intestine, gall bladder and urinary bladder.

B. Direct spreading of carcinoma

Most frequently it is observed in cervical carcinoma. Metastatic (or primary) peritoneal carcinoma growing into the uterine corpus is rare. Carcinomas of the colon and rectum, which have penetrated into the myometrium, were described. The continuous spreading of the carcinoma of the urinary bladder into the uterine cavity is rare.

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PETER STOLL

Heidelberg, Germany

I. General

Diagnosis of endometrial carcinoma is based more on the general architectural structure of the glands and the development of the endometrial stroma than on the cytological characteristics of individual cells. The tumor, in the beginning of its development, is confined to the endometrium and in most cases grows in a polypoid manner into the uterine cavity; destruction of the endometrium may be found only in later stages. Therefore, the diagnosis may be extremely difficult, especially when only scanty material of curettings is available for histological diagnosis. Furthermore, the location of the growth may be impossible if the clinician does not give accurate information as to whether or not the material comes from the uterine cavity or from the endocervix. A fractionated curettage of the endocervix and of the uterine cavity should always be done and sent to the laboratory properly labeled.

One should always try to work closely with the clinician in order to decide among:

adenocarcinoma corporis
adenocarcinoma cervicis
adenocarcinoma corporis et cervicis
metastatic adenocarcinoma corporis and/or cervicis (primary lesion in the ovary)

This differentiation is essential for the type of treatment.

II. Histological classification:

The histological diagnosis of adenocarcinoma also encounters difficulties in other directions, namely in connection with carcinosarcoma and mixed tumors, but especially in the differential diagnosis of benign functional atypia within the endometrium (mostly seen in certain cases of endometrial hyperplasia).

The following scheme is used in our laboratory:

1. Benign: Simple hyperplasia: Homologous

   Heterologous: Stromal hyperplasia predominantly / Glandular hyperplasia predominantly

   Cystic pattern

   Regressive type

   With secretory effect

   Atypical hyperplasia

2. Borderline cases:

   atypical hyperplasia with marked glandular atypia

   so-called adenoma malignum

   so-called adenocarcinoma in situ of the endometrium

3. Malignant: adenocarcinoma

   A. very well differentiated and highly organized

   B. well differentiated and organized

   C. poorly differentiated and less organized

   D. predominantly papillary structures

   E. more than 2/3 solid

   F. mucin production dominating

   G. adenoacanthoma, with squamous epithelial areas

   H. squamous carcinoma

   I. carcinosarcoma and mixed-tumors

This classfication is modified after Reuterwall (1), who in 1941 gave a competent classification for radiotherapeutic use. In our classification we are using 2 initials: the main diagnosis is given in capital letters and the second diagnosis in small letters. This is done to cover the variation of architecture in the different areas of the growth. In 447 cases of endometrial adenocarcinoma seen within the last 20 years we have found the following distribution of the types mentioned above:

	a	b	c	d	e	f	g	h	i	predominant type	pure type
A	11	8	1	2	3	2	2	-	-	29	11
B	6	84	49	49	40	17	5	-	1	251	84
C	-	1	20	13	30	-	1	-	1	66	20
D	1	9	9	28	6	3	-	-	-	56	28
E	-	8	5	-	14	-	-	-	-	27	14
F	1	-	-	-	1	1	-	-	-	3	1
G	-	3	-	-	-	-	9	-	-	12	9
H	-	-	-	-	-	-	-	1	-	1	1
I	-	1	-	-	-	-	-	-	1	2	1
										447	169

This table shows the puzzling variation of architecture in different parts of the tumor. Comparing these findings with the age of the patients, there is a slight increase of poor differentiation within the higher age-groups. There is also a slight decrease in the cure rate in the groups of poorly differentiated types as compared with the highly differentiated types.

Distribution of age: endometrial carcinoma: 447 cases

reproductive: 43	cure rate:	72.9%
climacteric: 55		66.7%
menopause: 349		52.8%

III. Adenocarcinoma of the endometrium and glandular hyperplasia:

In 447 patients with endometrial carcinoma, diagnosis of the functional stage of the endometrium was possible in 64 cases. Glandular hyperplasia was found in 4 cases within this group of 64 cases.

We failed to detect glandular hyperplasia preceding the development of endometrial carcinoma, and we feel, therefore, that hyperplasia of the endometrium as a functional episode in a certain age-group does not play an important role in the development of adenocarcinoma.

Seven hundred and ninety cases of glandular hyperplasia of the endometrium were studied since 1935 and the courses of the patients were followed up until 1957. Only 10 of these patients developed endometrial carcinoma within intervals of 1/1/2/10/11/11/12/12/13/17 years. Besides these cases, localization of cancer was later found in 28 cases (cervix: 9, mamma: 6, stomach: 7, brain: 2 ovary: 2, lung: 2).

Within the number of 790 cases of hyperplasia there were 24 cases with marked atypical hyperplasia. These patients received the following treatment:

curettage alone	8
hysterectomy	2
radium and/or X-ray	14

One of these patients developed cervical squamous carcinoma after 8 years, all others were discharged in good condition and are still living.

IV. Borderline Cases:

In borderline cases, if one cannot decide by a single curettage whether or not the atypia is malignant, one should not classify the case as true carcinoma. In an older woman, a hysterectomy, as a prophylactic procedure, is well indicated and also both ovaries may be taken out. I feel, however, it would not be a failure then to stop all kinds of hormonal treatment, while keeping these patients under control and close observation.

V. Histology and Cytology in Endometrial Carcinoma:

If one considers the difficulties in the histological diagnosis of malignancy even if one has the whole piece of tissue in multiple sections under the microscope, I would hesitate making up my mind on the basis of exfoliative cytology. Hyperplasia, as well as endometritis, exhibits cellular changes, which may be even more marked than in a well-differentiated adenocarcinoma. In a patient older than 35 years, with irregular uterine bleeding, curettage is indicated. This would not only be a diagnostic but also a therapeutic procedure. In such a case, malignancy can be ruled out, and one obtains valuable information for further hormonal treatment.

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DISCUSSION

ANTHONY F. ANDERSON, Edinburgh, Scotland, U.K.:

1. To Dr. Stoll: Altogether admirable, though his own conclusions do not seem to justify the elaborate classification in so far as it does not apparently lead to differences in treatment. I particularly agree with his last paragraph as general practitioners have already asked me if I will interpret smears from cases which they regard as needing "no more than a smear."

2. To Dr. Korte: What the patient needs and the surgeon hopes for is, a report which will guide his treatment. I believe Korte's descriptions are evidence of extremely thorough observation,

but wasted effort, as no two pathologists could possibly agree to this detailed extent. This detail leads young pathologists, in training at least, to a point where they cannot see the wood for the trees.

3. To Dr. de Brux: I wish he had pointed out that all fundal adenocarcinoma is in situ as far as the curettings are concerned, for the curette is not meant to produce myometrium, which alone can lead to opinions about invasion or otherwise. Far from being surprised at the outcome of his quoted case, it seems to me to support Hertig's contention to the hilt - just as invasive carcinoma of the cervix may eventually be demonstrated in the total hysterectomy specimen, although the biopsy only showed an in situ lesion.

HANS F. BETTINGER, Melbourne, Australia:

A. Classification:

I believe the classification of Korte, but also that of Stoll, is far too elaborate. In very large material, one will find a case for all the many sub-groups, but these divisions are, at best, only of academic interest. The number of cases in most of the sub-groups is far too small to allow a correlation with clinical features, radiosensitivity, post-operative results and long-term survival. The multitude of terms would only be confusing to the clinician and not of sufficient value to the pathologist. We have been using a classification very similar to de Brux's and have found it quite satisfactory.

B. Individual forms of carcinoma of the endometrium:

1. Age distribution: The statement of de Brux, that in his series of 250 cases only three occurred before the menopause, is not borne out by Stoll's figures and not by our material. In our last 6 years series of 180 cases, one woman was 27, five were in their thirties and another ten were in their forties, before the menopause.

2. Adenoacanthoma: This sub-group has not received in the literature, as well as in the contributions, the attention it probably deserves especially when the problems of the cytologist are taken into account. In our series of 180 cases, forty-two (24%) were adenoacanthomata. The frequently made claim that they are more benign than the classical adenocarcinoma is not borne out by an analysis of our figures.

3. Carcinomata of mesonephric origin: I believe the establishment of a separate group for these tumors, as suggested by de Brux, is fully justified but I cannot follow him at all when he refers to —

4. Atypias related to periovular vestiges, as I have not seen his photographs.

5. Carcino-sarcomas with a sarcoma of the endometrial stroma: This is a still more cumbersome term than "mesodermal mixed tumors." I doubt whether or not many readily realize that "chorion cytogene" of the French is just a particular expression for "endometrial stroma."

C. Metastatic Carcinomata

If tumor deposits are simultaneously present in the uterus and in one or both ovaries, far more consideration should be given to the suggestion by Huber and others that these tumors are not primary and secondary but multiple primary tumors. This is not only of theoretical interest. Its practical importance lies in the fact that with secondaries present, the prognosis must be bad, while with two primaries it should not be much worse than with one.

D. Hyperplasia and Endometrial Carcinoma

I fully agree with Stoll's contribution.

E. Carcinoma in situ

While I believe that carcinoma in situ of the endometrium occurs, we should be extremely cautious in the use of this term. Otherwise, we jeopardize the whole concept. At the cervix, carcinoma in situ is, at least in theory if not always in practice, a clear-cut condition. The epithelium shows all the cellular characteristics of a carcinoma, while invasion, in the material at our disposal, has not occurred. In the endometrium, the diagnosis of carcinoma depends, as de Brux and Stoll have pointed out and with whom I fully agree, far more on architecture and pattern than on cellular detail. In curettings, and this is the material on which most of our diagnoses are made, invasion can sometimes be suggested but never be excluded. The diagnosis of carcinoma in situ should therefore not be made on curettings, but only on the basis of an examination of the whole uterus. Even then a very large number of sections must unequivocally show that (a) the lesion has the definite pattern and cellular detail of a carcinoma and (b) that no invasion of the muscular wall has occurred. The number of such cases will be quite small and the delineation of such a finding from one of atypical hyperplasia will be frequently very difficult indeed.

CLOSING REMARKS

JEAN de BRUX, Paris, France:

In answer to Dr. Bettinger, I regret that my wording was not clear enough to be understood.

1. "Cytogenous chorion sarcoma": I agree that "stromal endometrial sarcoma is a better term, universally understood.

2. "Ovular vestiges": I believe Bettinger did not quite understand my statement. I was referring to the false pictures of endometrial carcinoma given by the endometrial reaction under chorionic stimulation of placental debris. These atypical endometrial pictures were described by Arias-Stella and later studied by Vaissade and myself.

3. I am in full agreement with Bettinger's statement about adenoacanthoma, but I wish to add this: The number of adenocarcinomas of the uterus before menopause is very limited. But their number increases proportionally with the number of cervical squamous carcinomas.

WOLFGANG KORTE, Bonn, Germany:

According to the title of the Symposium and to the title of my topic, the question was to give a short fundamental survey.

One must not withdraw from the obligation to only describe as long as one is not able to examine the progress of a disease in suitable objects of study.

I think it is indispensable, in any histomorphological paper which introduces cytological discussion, to point to the multiplicity of tissue pictures under one term of corporal carcinoma. Hence, it follows, how difficult it is to interpret cytological material, which has been taken or has been desquamated from the uterine cavity.

Classifications will necessarily produce opposition. I emphasized it in the beginning. The described histomorphological forms of carcinoma coincide with the recent studies known in European countries.

I believe the tendency to simplify biological problems too much is more dangerous for young pathologists, in training at least, than the necessity for some time to strive hard for the identification of various "single trees" in a "mixed wood."

I want to answer Bettinger, concerning the problem of classification, in a similar manner as I did Anderson: This paper does not intend to be a clinical-pathological summary for daily use. Such a resume should stand at the end of a Symposium as a leading paper.

In the pathological-anatomical usage of the German language the "Adenoacanthoma" is hardly ever found.

Carcinoma from remainders of the Ductus mesonephridicus (Wolffian duct) are so rare that I believe a separate subdivision is superfluous.

The question of the double-carcinoma has always been difficult and controversial up until today. If one believes that two primary malignant blastomas are present, why could there not be still more undetected primary tumors? The prognosis then is not better than with metastasizing carcinoma. I fully agree with Bettinger as to the term "carcinoma in situ" for the endometrium. The diagnosis of the carcinoma from biopsy material may be tied to the architecture of the tumor and to the texture of the tissue, histomorphologically seen. The term of malignant blastoma is still tied to invasion and destruction. Diagnosis is a question of experience, definitions are conventions according to the respective amount of knowledge.

I think that "mesodermal mixed tumors" is a somewhat incorrect term, not to mention the certainly illogical but appropriate and commonly used expression "carcino-sarcoma." The conception of mesoderm in the old interpretaion can hardly be supported nowadays. If one, however, retains the old germinal layer classification, then according to the traditional doctrine this kind of mixed tumor originates from tissues of two different germinal layers.

PETER STOLL, Heidelberg, Germany: No comments.

HISTOCHEMISTRY OF ENDOMETRIAL CARCINOMA

MANUEL GALBIS
Valencia, Spain

In 15 cases of endometrial carcinoma we have investigated the following reactions: the PAS reaction with its corresponding fermentations to differentiate glycogen and mucopolysaccharides (salivary diastase and hyaluronidase) (1, 2), the methyl green-pyronine staining for RNA (5) and the Gomori technique for alkaline phosphatase (3).

The results of these reactions as a whole, show a marked irregularity. The results, on the other hand, have a certain uniformity if they are grouped according to the degree of differentiation.

In the highly anaplastic adenocarcinomas, in which no definite glandular structure can be recognized, the PAS reaction shows a very irregular distribution; there are areas with marked positive reactions next to negative areas. This position reaction is not due to the presence of glycogen because it resists digestion by diastase and diminishes but does not disappear when incubated with hyaluronidase. All of the neoplastic cells are positive to the pyronine reaction and negative to the alkaline phosphatase.

In the more differentiated carcinomas, with a defined glandular structure, the results of the reaction are variable.

In general, the PAS reaction is more positive and has a more regular distribution in higher differentiated neoplasms. Its intensity varies from a weak staining of the apical areas of the cells to a deep staining of the whole cell. This distribution is more frequently seen in the more mature forms. The PAS positive substance is for the most part glycogen, as can be proven by its digestion with the diastase. The incubation with hyaluronidase produces only a very slight decrease of the stain. According to this, the accumulated substance would be mainly glycogen with a neutral mucopolysaccharide. However, some cases are devoid of glycogen. In the glandular lumen there is an almost constant positive PAS reaction, but its intensity and distribution is very irregular.

It is remarkable that the picture seen in the glandular distribution of the PAS reaction is sometimes quite similar to the one found in the normal secretory endometrium.

The distribution of the pyronophilic RNA is also markedly irregular. The pyronophilic reaction is less marked than in the less mature cases but is still positive in the majority of glands, although very changeable - from a deep stain throughout the entire epithelium to some apical traces (more frequent). Also, in some cases the picture is quite similar to the one seen in the normal secretory endometrium with an apical staining, basal emptiness and discharge of pyronophilic material into the glandular lumen. The stroma is consistently negative.

The same irregularity is found in the distribution of alkaline phosphatase. Some glands show an intense reaction throughout the entire epithelial height, while others show only a faint reaction in the apex. Finally, there are some cases that show a similar picture to the one found in the advanced secretory phase endometrium with complete negativity to the ferment. The stroma shows positivity only in the vessels.

In spite of the wide variability of distribution in the reactions investigated, some general considerations can be drawn: 1) the more immature neoplasms show only a deposit of neutral mucopolysaccharides and, more regularly, of RNA. 2) In a more mature neoplasm there is a production and accumulation of glycogen, neutral mucopolysaccharides, RNA and phosphatase, and in some cases the neoplasm shows a secretory pattern. The differentiation can be such that the picture is similar to the one found in the advanced secretory endometrium (production and secretion of glycogen, apical deposits and expulsion of RNA and negativity to phosphatase).

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Histochemical observations on endometrial carcinoma reported in this paper have been related to the three histological grades of malignancy, which we utilize in our laboratory in assessing the histological degree of malignancy of individual tumors. Chemical constituents of endometrial carcinoma cells have been histochemically defined and assessed for each grade, in a search for possible chemical characteristics that may be consistently augmented or decreased with increased grade of malignancy in endometrial carcinoma. Recent papers (1, 2) have correlated some of these determinations with the analysis of the same substances in the non-malignant endometrium.

METHODS AND MATERIAL

Glycogen and glycoprotein were localized by application of the PAS technique of McManus (3) and the diastase control reaction suggested by Pearse (4). Alkaline phosphatase determinations were made by following Gomori's method (5). Observations on the succinic dehydrogenase system utilized the method proposed by Rosa and Velardo (6). Fishman and Baker's method (7) was used to identify β-glucuronidase. Total liquid lipids were visualized by means of Baker's sudan black B method (8). Cholesterol studies were carried out by means of the Schultz and Lohr test (9). Ribonucleic acid (RNA) was evaluated by the methyl green-pyronin Y method. Modification of this method, as used in our laboratory, has been published (10). Details of the other methods listed have also been presented previously (2).

Forty-eight cases of endometrial carcinoma have been obtained for these histochemical studies. Sufficient material was not always available for the processing of all methods on every specimen.

RESULTS

Glycogen and Glycoprotein

These substances, when present, presented a most irregular distribution, as indicated in the figure captions. Glycogen occurred in large and small amounts or was absent in different areas of the same tumor. In some tumors it was only minimally present. It was cytoplasmic and luminal in location. Glycoprotein, presumably mucin, showed a similar variation in distribution. Its site was predominantly at the apical margins of the cells and in the lumina. Approximately one-third of the cases studied were devoid of both glycogen and glycoprotein, some specimens presenting a patchy distribution of one or the other constituent exclusively. Stromal glycogen was observed in small amounts in a few tumors. The variable distribution of these substances occurred in all grades.

Alkaline Phosphatase

The figures present the alkaline phosphatase reaction as observed in specimens of the three grades of malignancy. Localized sites of this enzyme appeared to decrease with loss of differentiation in these endometrial tumors although it was variably present or absent in all grades. The site of its occurrence indicates its presence as related to the function of secretion rather than to the phenomenon of malignancy.

Succinic Dehydrogenase System

All specimens reacted for this enzyme system demonstrated reacting structures in the cytoplasm of viable epithelial cells. Stromal cells appeared to show less activity of this enzyme system than the glandular tissue. Necrotic areas in tumors presented a negative response to the reaction. Comparison of the figures representing the reaction in differentiated and undifferentiated tumors indicates that the diformazan deposits occurred in similar amounts and types regardless of the histological grade of malig-

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nancy. These observations based on histochemical methods, however, cannot assess the activity of such an enzyme system.

β-Glucuronidase

Since the initiation of the histochemical study of this enzyme in our laboratory, only two specimens of endometrial carcinoma have been obtained. These current observations, therefore, are merely preliminary. The figures illustrate that the cytoplasm of endometrial carcinoma cells presented a somewhat intense reaction for this enzyme with a minimal response in the stroma.

Lipids

Abundant sudanophilic lipid was observed in the stroma of endometrial carcinoma. The distribution of stromal lipid was frequently patchy in the same tumor section. Since differentiated tumors contain more stroma than undifferentiated do, more stromal lipid was visualized in the latter. The lipid droplets were cytoplasmic in distribution and occurred in both rounded and elongated cells of the tissue. Much of this stromal, liquid lipid gave a positive response to the cholesterol test. Glandular lipid was present to a lesser extent among the tumors. In the sections reacted for sudanophilic lipid, it was either absent or variable in distribution. The epithelial lipid in some tumors responded to the cholesterol test.

Ribonucleic Acid

RNA studies have been based primarily on the identification of nucleoli in interphasic nuclei, since it is known that the nucleolus is the site of RNA at that phase of the mitotic cycle. Nucleoli occur as single and as multiple structures. Figures 30 to 38 are presented to illustrate that, with increased grade of malignancy, 1) single nucleoli per nucleus showed increase in size, 2) more multiple nucleoli per nucleus occurred and 3) more irregularity of nucleolar form was observed. It should be emphasized that all illustrations in this group were taken at the same magnification. Tables of nucleolar counts and measurements have been presented in other publications (1, 2). Eight thousand nuclei were observed for multiple nucleolar counts and the diameters of single nucleoli were measured in 2,400 nuclei. The biometric data in the tables indicates that there was a statistically significant difference in nucleolar counts and measurements between Grades I and III. Parenthetically, a similar significant difference was established between the non-malignant and malignant endometrium.

Cytoplasmic and chromosomal RNA was observed in all tumors. Comparison of cytoplasmic RNA in Grade I tumors (Figs. 30 and 33) with that in a Grade III tumor (Fig. 38) indicates that in some Grade III cells more cytoplasmic RNA subjectively could be observed than in some cells of differentiated tumors. It has also been noted that the nucleoli of endometrial carcinoma cells in peritoneal fluid (Fig. 40) tend to resemble their counterparts in tissue sections of hysterectomy specimens (Fig. 39).

COMMENTS AND SUMMARY

In this series of endometrial carcinomas, glycogen, glycoprotein, alkaline phosphatase and sudanophilic lipids presented the irregular distribution reported by others (11, 12). Neither these substances nor succinic dehydrogenase, β-glucuronidase and cholesterol were observed to be consistently augmented or decreased with increasing histological grade of malignancy.

RNA analysis of nucleolar population demonstrated nucleolar alterations which may imply an increase in RNA with loss of differentiation in endometrial tumors. Larger and more multiple nucleoli per nucleus are not necessarily an indication of increased nucleolar RNA content. However, phase microscope observations (13) have correlated, in other material, such nucleolar alterations with increased nucleolar activity and concomitant increased production of cytoplasmic RNA. Cytoplasmic RNA was consistently present in all grades of the current series of endometrial tumors. The qualitative observations, dependent on the technical method used, do not permit any conclusion concerning its increase with loss of differentiation.

However, the nucleolar alterations, which have been demonstrated to occur with increased grade of malignancy, may serve as aids in differential diagnosis and for prognosis.

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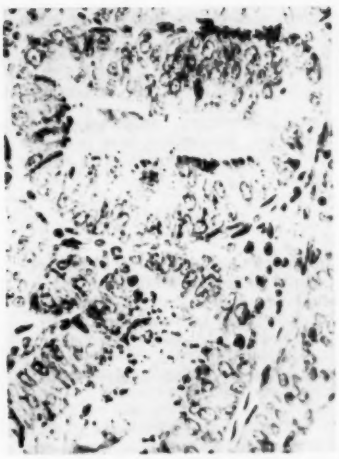


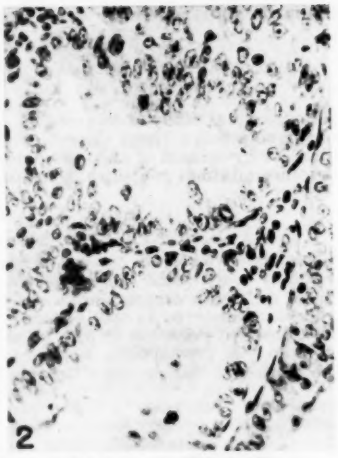


Fig. 1. Glycogen in Grade I may be distributed diffusely throughout the cytoplasm and may be observed in glandular lumina, as shown, or may be absent even in adjacent glands. No stromal glycogen was present in sections of this tumor.

Fig. 2. Diastase control section of Fig. 1. No glycoprotein was present in these glands.

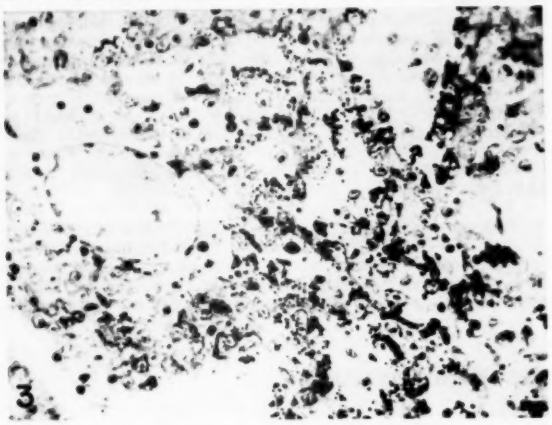


Fig. 3. Abundant glycogen in area of Grade III tumor. Adjacent area to left was devoid of glycogen.

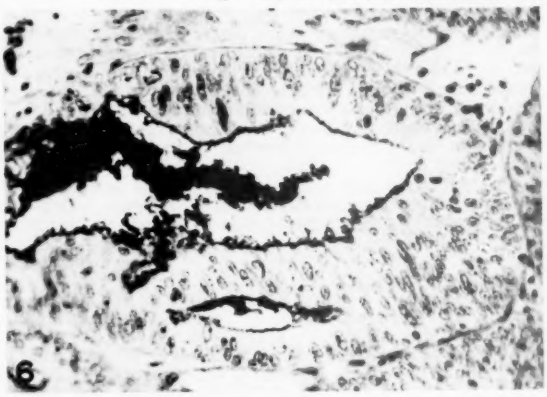
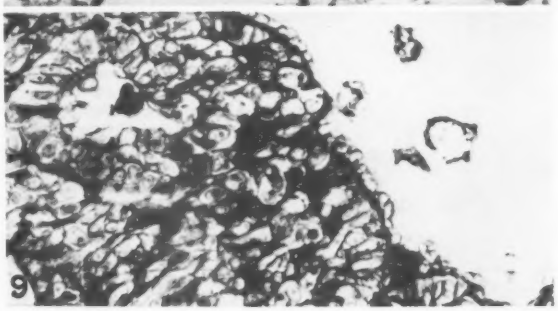


Fig. 4, 5, and 6 represent diastase control sections of Grades I and II tumors to demonstrate glycoprotein. Glycoprotein, when present, was observed at the apical margins of the glandular cells, in the lumina (Fig. 6) and occasionally in the cytoplasm of epithelial cells (Fig. 5). Marginal glycoprotein as shown in Fig. 4 was absent in other glands of the same tumor (Fig. 2).



Figs. 7 and 8. Alkaline phosphatase in sections of Grade I tumors. The reaction, more intense in Fig. 8, occurred irregularly at the luminal margins of the epithelial cells and in the glandular lumina. The presence of this enzyme was not demonstrated in numerous glands of these tumors. Note excellent reaction of arteriolar endothelium in all photomicrographs presented for demonstration of alkaline phosphatase.

Fig. 9. Sections of this Grade II tumor showed an unusual distribution of alkaline phosphatase in addition to that noted above. The limiting membrane of cells throughout the multilayered epithelium presented the reaction. Stroma was negative for the enzyme with intense arteriolar reaction.

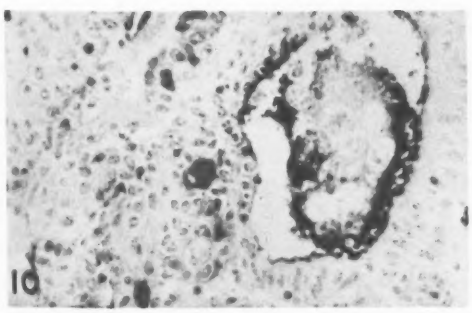


Fig. 10. Alkaline phosphatase predominantly was absent in the glands of this Grade II tumor except in the lumina and margins of some dilated glands and in small lumina-like spaces.

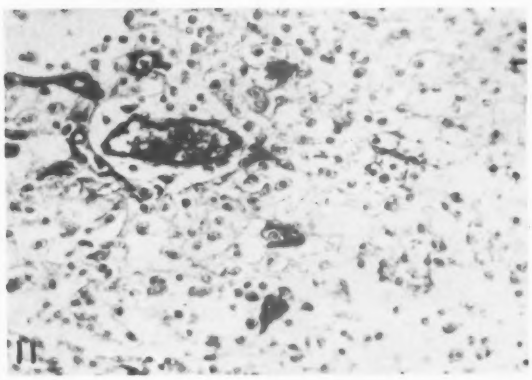


Fig. 11. Grade III tumors, composed of solid sheets of epithelial cells, reacted for alkaline phosphatase only in the vessel endothelium and in some tiny spaces where the cells were not contiguous.

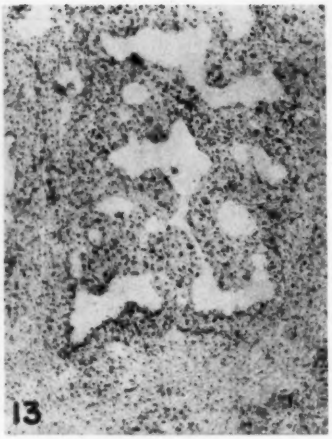
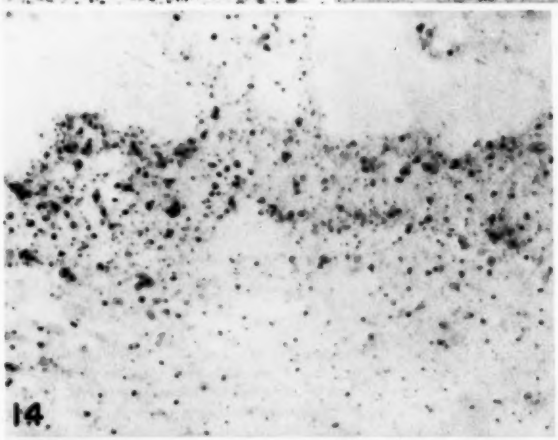
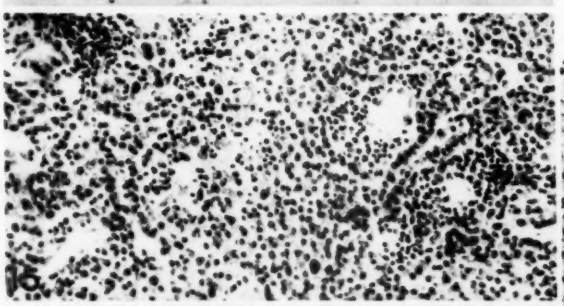
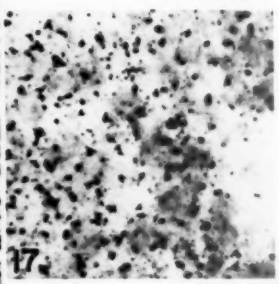


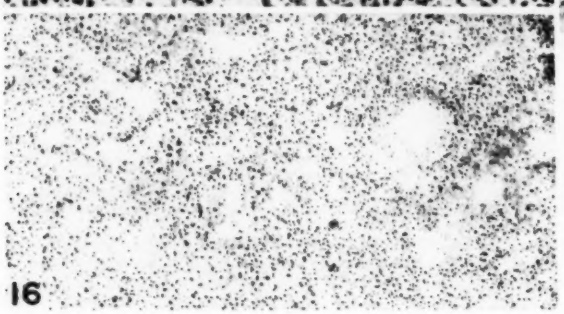
Fig. 12 and 13. Successive sections of a Grade I tumor. (Fig. 12) Hematoxylin and Eosin. (Fig. 13) Representation of activity of succinic dehydrogenase system which presented a somewhat stronger reaction in the glandular epithelium than in the stroma. Note the absence of reaction in the glandular lumina and in the vessel shown at upper left of the figures. Fig. 14 presents a higher power view of the epithelium and stroma at the base of the gland shown in Fig. 13. Oval spaces in the epithelium represent unstained nuclei. The reacting structures were cytoplasmic in distribution and were composed of single and multiple granules and rods of diformazan.

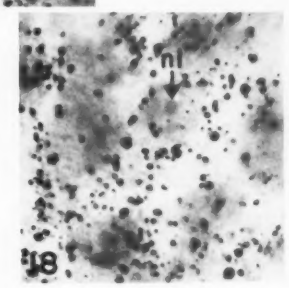


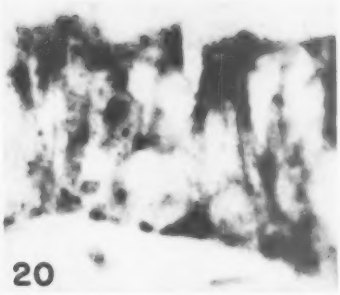
Figs. 15 and 16. Successive sections of a Grade III tumor (Fig. 15) Hematoxylin and Eosin. (Fig. 16) Succinic dehydrogenase system. The reaction was demonstrated throughout solid epithelium of the tumor. It was absent in vessels and negligible in thin strands of stroma. Fig. 17 shows a higher power (x 684) or area to left of largest vessel in Fig. 16. Fig. 18 (x 918) represents the reaction in the whole cells of a tissue smear of the same tumor. Unstained nuclei and nucleoli may be visualized at these higher powers (nl = nucleolus). The irregular appearance of the reacting structures is due to the multiplicity of granules of which they are composed.

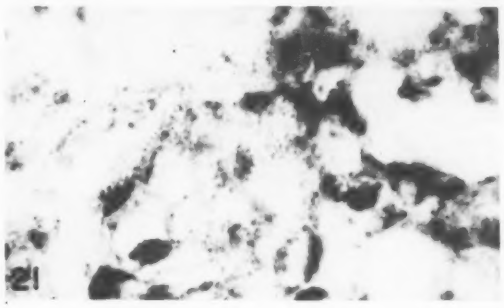












Figs. 19, 20 and 21 represent sites of activity of β-glucuronidase. Light, oval spaces in the epithelium denote nuclei. Fig. 19 is a low power view taken from a Grade I tumor, Fig. 20, a higher power (x 918) from the same section. The reacted structures, predominantly small granules, are distributed throughout the cytoplasm of the epithelial cells and, to a lesser extent, in the cytoplasm of stromal cells. Fig. 21 shows reacted structures in a Grade II tumor, (x 918). Variations in intensity may be due to imperfections in technique.

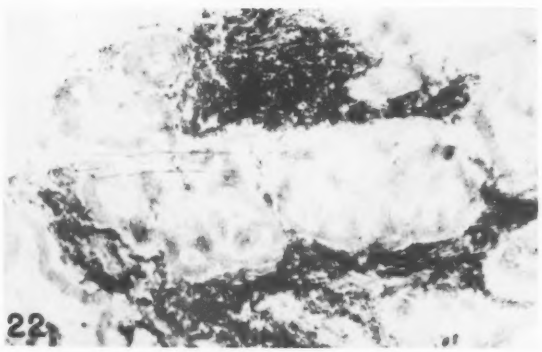


Fig. 22. Sudanophilic lipid in the stroma of a Grade I tumor. The glands of this tumor were negative for lipid with the exception of its occurrence in infiltrating leukocytes and histiocytes. Fig. 23 shows the cholesterol reaction in a section of the same area of the same tumor. Cholesterol was present in droplets similar to those that responded to the sudanophilic lipid test. The impression that more cholesterol was present than total sudanophilic lipid is misleading. More stroma was present in Fig. 23.

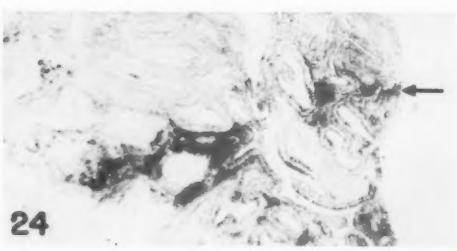


Fig. 24. Sudanophilic lipid is another Grade I tumor. Fig. 24 is presented at a much lower power to emphasize that such lipid was irregularly present in the stroma of tumors. Some glandular lipid may be visualized even at this low magnification at the right of the figure, whereas glands in other areas were devoid of lipid droplets.

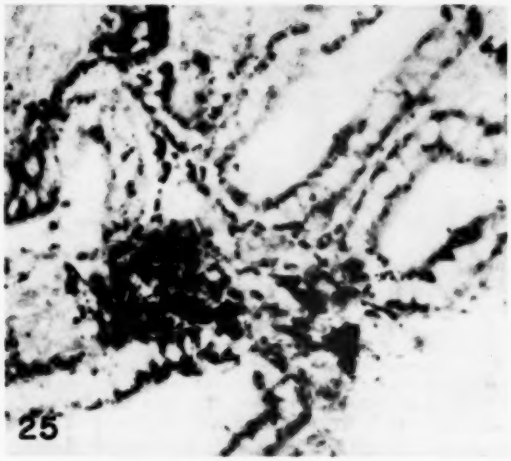


Fig. 25. Sudanophilic lipid in area indicated on previous figure. Nuclei were not stained. Lipid was visualized in the cytoplasm both basally and apically in epithelial cells and in stroma cells in the form of discrete lipid droplets.

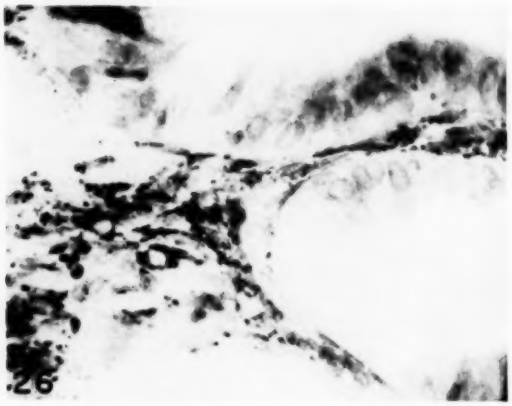
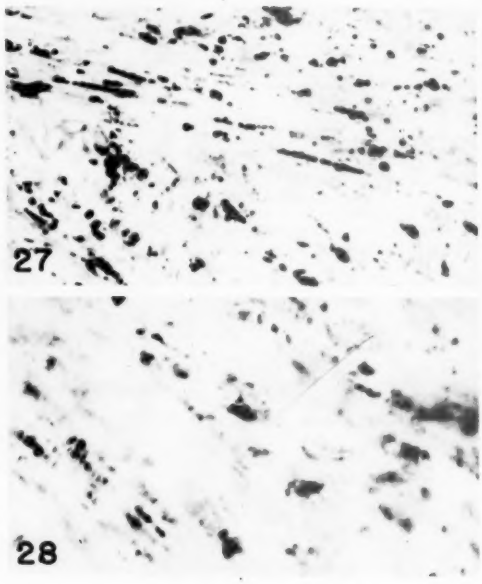


Fig. 26. Lipid was not observed in the glandular epithelium of this Grade I tumor and was present in scant amounts in the stroma. Many of the lipid-filled stromal cells in this tumor were rounded in appearance.

Fig. 27. Stromal sudanophilic lipid in a Grade II tumor. The lipid was present predominantly in the cytoplasm of elongated connective tissue cells. Fig. 28 represents the cholesterol reaction in another section of the same area as shown in Fig. 27. Some of the sudanophilic lipid also reacted positive for cholesterol.



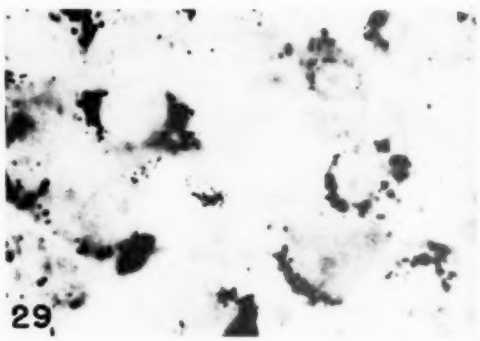
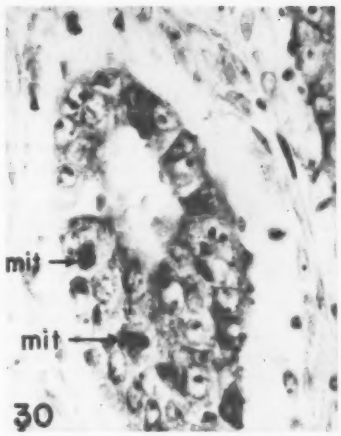


Fig. 29. Sudanophilic lipid in the epithelial cells of a Grade III tumor (x 918). Unstained nucleoli may be visualized. Some of these lipid droplets responded to the test for cholesterol.

Figs. 30 to 38. Ribonucleic acid (RNA) in sections and tissue smears of endometrial carcinoma. All photomicrographs in these figures were taken at the same magnification, (x 918).

Fig. 30. Section of a Grade I tumor. Pyroninophilic nucleoli markedly stand out in the pale oval nuclei. Note the relatively large single nucleoli per nucleus and the occurrence of multiple nucleoli. Cytoplasmic RNA is diffusely distributed throughout the cytoplasm of the epithelial cells. The chromosomal RNA, imperfectly illustrated in black and white figures, appears as a diffuse halo around the methyl-green reacted chromosomes of the metaphase mitotic figures.



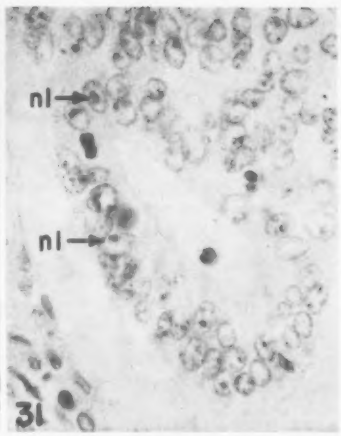
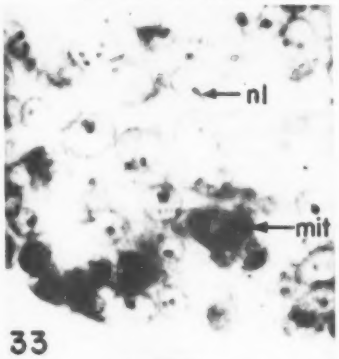


Fig. 31. A successive section of the same gland shown in Fig. 30. This section was subjected to ribonuclease digestion before application of the staining reaction. All pyroninophilic RNA was removed by the procedure. Note the DNA rim of the clear single nucleoli and the absence of cytoplasmic RNA. The metaphase figure appears more distinct than those in Fig. 30 because of the extraction of chromosomal RNA.



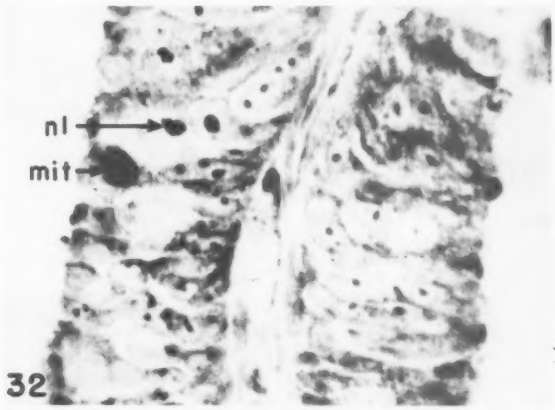
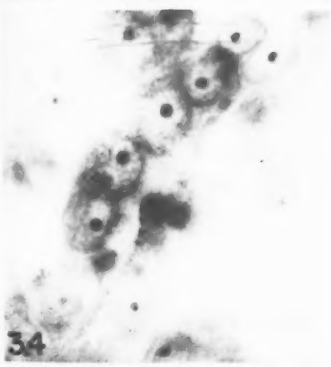
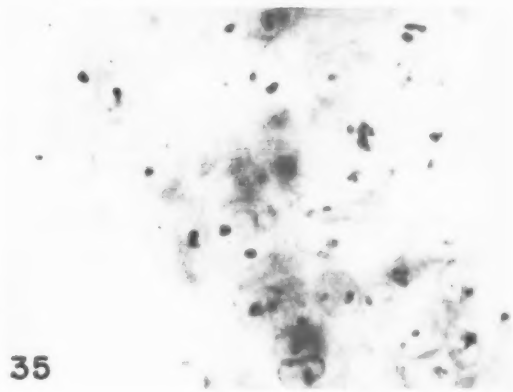
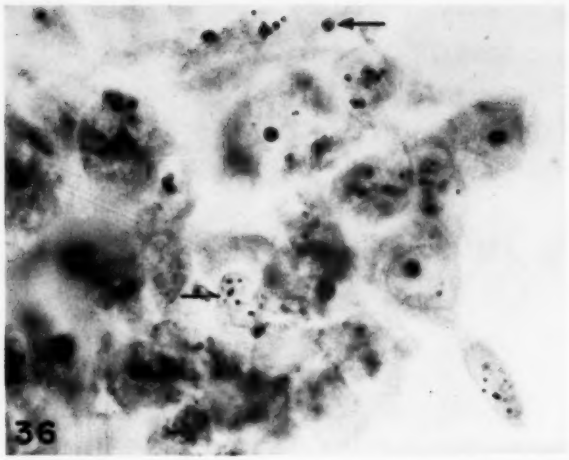


Fig. 32. Section of a Grade II tumor. RNA has the same localization as in Fig. 30. Single nucleoli per nucleus are larger and nucleolar bodies tend to present a more irregular form in less differentiated tumor cells.





Figs. 33, 34 and 35. Tissue smears of Grade I and Grade II tumors. Tissue smears present whole nuclei and cells for RNA analysis. Fig. 30, Grade I, demonstrates single and multiple nucleoli per nucleus. An elongated nucleolus is designated "nl." This preparation is predominantly composed of naked nuclei so that cytoplasmic RNA is inadequately shown except in the area with the mitotic figure. Figs. 34 and 35, Grade II, exhibit larger single nucleoli per nucleus and more multiple and irregular nucleolar structures than in Fig. 33. Note that the sizes of large single nucleoli in the Grade II tissue smear (Fig. 34) and section (Fig. 32), correspond.



Figs. 36, 37 and 38. Tissue smears of Grade III tumors. Fig. 36 presents the nucleolar RNA status characteristic of undifferentiated tumors. With increased grade of malignancy, nucleolar populations show increased size of single nucleoli, more multiple nucleoli per nucleus and greater morphological variations. Numerous nuclei in undifferentiated tumors demonstrate the nucleolar characteristics of differentiated tumors. Compare the nuclei indicated in Fig. 36 and those in Fig. 38 with nuclei in Figs. 33 and 35. However, the augmentation of nucleolar size, numbers and irregularity, exhibited in undifferentiated tumors (Figs. 36 and 37), has never been observed in differentiated tumors.

Fig. 38. Three cells in the metaphase stage of mitotic division. The metaphases themselves are reproduced too darkly for clear chromosomal delineation. Focus was on the lower cell in the figure and clearly shows the cytoplasmic limits of the cell and its cytoplasmic RNA.

Fig. 37. A large, naked nucleus with abundant pyroninophilic substance, somewhat diffuse in form. The large vacuoles have DNA rims and contain scant amounts of RNA reacting material.

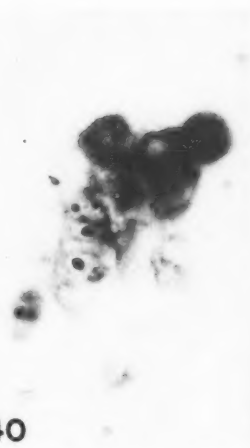


Fig. 39. Section of endometrial carcinoma located in a hysterectomy specimen. The routine Hematoxylin and Eosin staining of this section morphologically shows the nucleolar characteristics of malignancy. Ten months after hysterectomy, ascites fluid was obtained from the same patient at a second laparotomy. Fig. 40 shows cancer cells from the ascites fluid. These cells were reacted cytochemically for RNA. Note the similarity of nucleoli in the two figures.

DISCUSSION

CONSTANTIN HEROVICI, Villejuif-Paris, France:

The extensive work of Margaret Long is remarkable for its precision and also for its sensible conclusions. I should like to make two remarks:

The first is technical and concerns the methods used for showing the presence of RNA. The method using methyl-pyronine green is sufficiently specific for the RNA of the cytoplasm which stains red, but it does not very readily differentiate between the cytoplasm having a high RNA content and that having a low RNA content, as they are always more or less red. This may be remedied by using a cytophotometer or simply by adding orange G to methyl-pyronine green, which colors the cytoplasm of low RNA content yellow and the cytoplasm of high RNA content red by creating a quantitative threshold for the tinctorial affinity of pyronine.

My second remark is even more doctrinary. If it is true that an increase of nucleolar RNA is evidence of the hyperactivity of the cells and, therefore, perhaps of increased malignancy, it is also true that the unhealthy cells which are dying through degeneration or necrosis, or even the after effects of irradiation, show an identical increase in nucleolar RNA. It seems to us that to be able to interprete the increase of RNA correctly, as proof of a super-malignancy, it is indispensable that the total quantity of nuclear DNA remain absolutely intact or even be increased.

Galbis, in an equally interesting presentation, compared the cytochemical characteristics of a tumor to the degree of differentiation of the uterine adenocarcinomas.

It seems to me that perhaps it might have been useful also to take into account the carcinomas developed from squamous metaplasia, the adeno-acanthoma, which is so often observed in daily practice, and to try to determine whether the rather spatial way in which it evolves has any relationship with its cellular metabolism.

WALTER SANDRITTER, Frankfurt am Main, Germany:

To Dr. Galbis: I would like very much to know how the material used in the reaction for alkaline phosphates is prepared (frozen section?). The fact that the reactions are so frequently made at different time intervals after removal and in addition, that variations in section thickness can simulate a stronger or a weaker reaction, results, to my way of thinking, in considerable difficulty.

To Dr. Long: I would be greatly interested in knowing if the cancerous tissue behaves differently (histochemically considered) than normal tissue.

CLOSING REMARKS

MANUEL GALBIS, Valencia, Spain:

All our material for the alkaline phosphatase determination has been fixed in absolute alcohol or acetone at 4° C. for a duration of 24 hours. Certainly management and thickness of tissues influence the reaction intensity and diffusion. We prefer paraffin embedding and we cut tissue in 5 - 7 μ sections.

Of course, it would be interesting to do histochemical research on the adeno-acanthoma, but these tumors are infrequently present in our material and one has never been properly fixed for histochemical procedures.

We have found adenocarcinomas in which morphological differentiation goes together with a functional, histochemical differentiation. We can not assert whether the histochemical differences are related to olinical evaluation or due to the fact that our cases are scarce and recent.

MARGARET E. LONG, New York, New York, U.S.A.:

I am happy to reply to Sandritter's query concerning histochemical differences between normal and malignant endometrial tissues. The limitation of the title of the principal paper precluded the consideration of similar histochemical observations on our control series which included seventy-seven cases of non-malignant endometria from hysterectomy specimens and thirty endometrial biopsies obtained from women with known normal menstrual cycles.

In contrast to the variable distribution of glycogen and glycoprotein in endometrial carcinoma, these substances appear in increasing amounts during the progression of the normal menstrual cycle. Except during the proliferative phase, my observations indicate that there is less glycogen and glycoprotein present in endometrial carcinoma than in normal endometrium. Subnuclear glandular glycogen and epithelial tip and luminal glycoprotein increase slightly during the proliferative phase. The presence of large deposits of glandular basal glycogen and of stromal glycogen characterizes the start of the secretory phase. As the secretory phase progresses, glycogen appears throughout the epithelial cytoplasm, then successively accumulates in supranuclear and luminal sites. The presence of glycogen is notable in

predecidual cells as they make their appearance, as well as in non-decidualized stromal cells. Glycoprotein becomes an increasingly prominent component of the luminal secretion during the secretory phase.

The alkaline phosphatase reaction, also variably present or absent in the epithelial cells of endometrial carcinomas, is visualized as increasing in intensity at the apical tips of the luminal epithelia and in the luminal secretion as the proliferative phase progresses. During early secretion, the apical tips show a decreasing response to the reaction, whereas the luminal secretion response is intensified to a peak during the mid-secretory portion of the cycle. The glands of the normal late secretory endometrium show a decreasing to negative response to the alkaline phosphatase reaction. Arteriolar endothelium is positive in both normal and malignant endometrium.

Reactions for the succinic dehydrogenase enzyme system and for the enzyme β-glucuronidase show no differences between normal and malignant endometria. It must be remembered, however, that histochemical observations on enzyme systems are not necessarily an evaluation of enzyme activity.

Sudanophilic lipid as well as one of its component substances, cholesterol, was observed in greater abundance in the stroma of endometrial carcinomas than in the stroma of normal endometria. Scattered proliferative stromal cells were positive for lipid. Lipid was variably abundant in the stromal cells of secretory endometria as well as in the glandular epithelium. Predecidual cells contained sudanophilic lipid, some of which reacted positively for cholesterol. There was no observable difference in the glandular lipid content between normal and carcinomatous tissues.

Analysis of nucleolar conditions in normal endometria, based on the ribonucleic acid content of nucleoli, demonstrated significant differences in size, number and morphology between the nucleoli of normal and malignant tissues. In the normal endometrium, single nucleoli per nucleus were smaller, multiple nucleoli per nucleus were less numerous and more regularity of nucleolar form was observed than in the malignant tumors. The nucleoli of the proliferative phase were the largest of the normal tissues, with those of secretory and postmenopausal types being successively decreased in size. Cytoplasmic RNA appeared to be more prominent in cells of the proliferative phase than in other non-malignant endometria. In turn, there seems to be more cytoplasmic RNA in carcinoma cells than in normal proliferative epithelium.

There seems little doubt that pyronin Y, used in conjunction with ribonuclease tests, is specific for RNA. The subjective type of observation inherent in histochemical studies, however, does not permit definitive quantitative conclusions. It was stated in the main paper that larger nucleoli do not necessarily contain more RNA than smaller nucleoli. It is of interest, however, that quantitative biochemical analysis of RNA in normal endometrium has shown a much higher RNA content in proliferative phase tissues than in secretory phase (1). A presumption then may be entertained that the larger nucleoli and apparent increase in cytoplasmic RNA in carcinomatous tissues may indicate that endometrial carcinoma cells contain more RNA than the epithelial cells of non-malignant endometria.

I should like to thank Herovici for his thoughtful and interesting discussion. I have had no experience with the orange G-methyl green-pyronin Y method for subjective determination of differences in RNA cytoplasmic content. My approach to a visual estimation of RNA differences among the several types of control and malignant endometria has been to mount sections of proliferative, secretory, atrophic endometrium and endometrial carcinoma of Grades I, II, and III all on the same slide. Sections of these different endometria then have exactly the same treatment in the course of the methyl green-pyronin Y reaction. On such a slide the tinctorial affinity of pyroninophlic RNA increases in endometrial types in the following order: atrophic, secretory, proliferative, Grade I, Grade II and Grade III.

In considering Herovici's second remark, I should like to express an opinion that augmented nucleolar size and multiplicity with increased grade of malignancy may be explained on a cytological basis by effects of the mechanisms of polyploidization. It is well known that regular or euploid and irregular or aneuploid chromosome duplication occurs in cancer cells. An explanation of the large nucleoli of Grade III tumor cells is that they represent a fusion of the nucleoli of duplicated nucleolar-bearing chromosomes. It has been my experience and has been reported by others (2) that nucleoli become smaller and disappear in the pyknotic nuclei of degenerating and necrotic tumor tissues. It seems to me that the increase in nucleolar size, and presumably in RNA, following irradiation is not identical with the increased size of nucleoli in non-irradiated malignant cells. The latter may be considered viable cells which are capable of further division; the former are more likely to be on their way out. DNA studies have not been included in the main paper and I prefer at this time not to comment on the DNA status of endometrial tumors.

In our histochemical studies of endometrial carcinoma, the adenocarcinomas with acanthomatous changes were not treated separately.

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CYTOMORPHOLOGY OF NORMAL ENDOMETRIUM

HANNS-WERNER BOSCHANN
West-Berlin, Germany

In the cytological smear obtained from the uterine cavity either by aspiration or the brush technique, we find, under normal physiological conditions, the following:

1. Cylindrical epithelia

   These are desquamated from the surface of the endometrium.

2. Glandular epithelia from the functionalis

   These cells show cyclic changes, thus, enabling us to recognize the hormonal situation from certain established criteria, which are less definite in cells from the gland orifice than in cells from the middle portion of the glands.

3. Glandular epithelia from the basalis

   They show very few cyclic alterations and always appear to be in a state of proliferation because of the dense, firm arrangement of the nuclear chromatin.

4. Stroma cells from the compacta

   In the second half of the menstrual cycle, as well as in normal or ectopic pregnancy, these cells show decidual changes.

5. Stroma cells from the basalis

   These cells always have nuclei rich in chromatin. They lie close together and because of this, one questions the effect of estrogen at all. This applies especially to those cases where these groupings are the only ones to be found.

6. Leukocytes, erythrocytes, histiocytes and mucus

In detail:

1. Cylindrical epithelia

   a. Single desquamated cells:

      The slender cellular body of cytoplasm is pushed outwards by an oval nucleus, which is sharply demarcated from the cytoplasm and contains abundant, finely granulated chromatin with some chromo-centers.

   b. Desquamated cells in clusters:

      One sees palisade-like cells which are, generally speaking, flatter than the cells from the glandular tubules. In the course of the proliferative phase they increase in length, while in the secretory phase they become smaller. However, these cells show almost no evidence of secretion.

In the senile age group, one sees a series of cuboidal cells. The cells form a honeycomb pattern when viewed from the superior or the inferior surface. Often one sees the glandular opening as a circular hole.

This uniform pattern is formed because, under normal physiological conditions, only very slight changes occur in the chromatin content or in the size or shape of the nuclei.

2. Glandular epithelia from the functionalis

   a. These cells are similar to those described under #1, but are somewhat longer (20 to 25 μ).

   b. They show more significant cyclic changes: The nucleus, which is slender and oval during the postmenstrual periods, becomes more rounded, and the cytoplasm becomes somewhat wider during the proliferative phase.

   During the course of the menstrual cycle the nuclear surface ranges between 31 and 53 μ^2 and averages about $40.7 + 5.9\ \mu^2$. During the proliferative phase we see mitoses and increased loosening of the chromatin which was previously very dense. Following the 10th or 11th day of the cycle, plasma vacuoles can be observed. Therefore, they are not a specific sign of the secretory phase! During the secretory phase, flattening and finally a decrease of the cytoplasm, by secretion, are seen. Thus, only free nuclei are found in the smear prior to menstruation.

3. Glandular epithelia from the basalis

   Similar to #2, in the early proliferative phase slender cell bodies are pushed out by sharp-edged, chromatin-rich nuclei.

4. Stroma cells from the compacta

   a. Roundish cells with a narrow margin of cytoplasm. They are either single cells or exist in more or less loose clusters depending upon the hormonal situation.

   b. In the proliferative phase the chromatin of the round nuclei appears more compact. On the other hand, in the secretory phase the chromatin appears less compact. In the proliferative phase the nuclei are adjacent, often show mitoses and, in addition, are spindle-shaped. During the late secretory phase the nuclei diverge from one another. The cellular borders become marked and the cytoplasm increases in size. The cells take on an epithelial-like appearance which is particularly marked during pregnancy and abortion.

   c. There is a gradual transition towards a histiocyte-like picture. Stroma cells lying in union may be very easily mistaken for glandular cells, especially if they are seen from the inferior or superior surfaces. For distinct identification we use cells lying at the margin and presenting a side view.

5. Stroma cells from the basalis

   Similar to #4 during the early proliferative phase.

6. Leukocytes, erythrocytes, histiocytes and mucus

   Principally, there is no difference from those found in vaginal smears. It might be mentioned that in some instances there are confluent transitions from histiocytes to stroma cells. The penetration into the stroma cell network by white blood cells causes one to think of endometritis.

Confronted by such a multitude of different cellular elements from the uterine cavity, it would seem to us that it would be an unjustified simplification to speak merely in terms of "endometrial cells." In the literature this term usually means clusters of cells, easily recognizable as glandular, but which are smaller than endocervical cells.

The specific identification of the original source of a single cellular element is possible when we find an easily recognizable cluster of cells lying close at hand.

Technique of Experiments

The material consisted of 567 extirpated uteri. Smears were taken and curettages performed from these uteri. Material for both kinds of studies (histological and cytological) were taken from the same spot in each uterus. Two thousand, two hundred and seventy slides were prepared in this manner. These "guided" smears were then compared to those taken preoperatively by aspiration and brush techniques.

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DISCUSSION

HANNAH PETERS, Bombay, India:

Boschann stresses that it is not possible to talk about "endometrial cells" in general. The columnar cells of the endometrium undergo constant changes during the course of a cycle. Their nuclei, which in the early proliferative phase stain lightly and are surrounded by distinct, prominent nuclear membranes, change in the later proliferative phase and become deeply stained, and chromatin clumps can be seen. During the secretory phase the cells enlarge at first and show cytoplasmatic vacuoles, whereas in the later part of the secretory phase the nuclei show various, characteristic types of degeneration (the "burnt-out" and the "shell-like" nuclei) (1). On the whole, the endocervical cells are larger than the endometrial cells. This, however, becomes a difficult criterion to evaluate in certain cases. In endometrial hyperplasia, for instance, the nuclei of the columnar cells may have diameters of 12 μ. I agree that the differentiation of endocervical and endometrial cells is often possible when these cells are found in groups or tissue pieces, but in the case of single cells I would hesitate to make such a distinction with any degree of certainty.

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CLOSING REMARKS

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

I would like to thank Hannah Peters for her completion of the discussion. I know her outstanding work concerning endometrial cytology, which I was able to discuss in personal talks with her during the Brussels' meeting in July, 1957. I am glad to be in full agreement with her present remarks. The difficulty of differentiating endometrial hyperplastic cells from endocervical cells, although existing theoretically, can be eliminated by a technique allowing the collection of pure endometrial cells without contamination by cervical mucus. Besides, as Hannah Peters stresses, the identification of single cells is always facilitated by considering whole groups of the same type, a principle which should be observed not only in endometrial cytology.

CYTOCHEMISTRY OF NORMAL AND ABNORMAL ENDOMETRIUM

MANUEL GALBIS
Valencia, Spain

In 42 morphologically normal endometria, from women with normal ovarian function, the cytochemical distribution of the following substances has been investigated: determination of polysaccharides with PAS reaction together with the corresponding fermentation to differentiate the glycogen (salivary diastase), acids and neutral mucopolysaccharides (hyaluronidase). The RNA is investigated by the pryoninophilic stain with methyl-green pyronine, the alkaline phosphatase by the Gomori technique.

Polysaccharides: In the proliferative follicular phase the glandular epithelium shows a weak positivity to the PAS reaction, which is limited to a line on the apex of the cells and a fine line somewhat more intense in the basal area. This distribution varies little during the entire phase. In the secretory phase a rapid increase of PAS positive substance takes place, which extends and occupies the whole height of the cell. In this phase, mainly in the middle secretory, expulsion of PAS positive substance towards the glandular lumen is observed. In the lumen a positive reaction can be seen. Towards the end of the cycle, just before menstruation, the cellular content of PAS is reduced; a number of cells only show small traces at the apices or are absolutely negative. The lumen continues to be positive. Incubations with diatase and hyaluronidase permit the isolation of the different components of this PAS positive substance.

Glycogen only appears in the glandular cells in the advanced proliferative phase, increases considerably in the secretory phase and diminishes in the premenstrual phase. Acid mucopolysaccharides which can be eliminated by hyaluronidase are present in the base of the cell during the whole cycle to diminish or disappear in the late secretion. They are barely positive in the apex. Neutral mucopolysaccharides (possibly glycoprotein) present a weak reaction localized at the apex of the cell in the proliferative phase, increase in the secretory phase; but continue to be limited to the apex. In the lumen they are positive but very scarce.

The cells of the basal glands also present a mild positive reaction to PAS that experiences no obvious changes during the cycle. In the stroma there is a positive reaction in the intercellular net which can be eliminated by hyaluronidase. The stromal cells in the secretory phase give a positive reaction due to the glycogen.

RNA: The cytoplasm of the glandular cells during the proliferative phase shows a strong pyroninophilic marked uptake of pyronine reaction in the whole height of the cell. During the early secretory phase the reaction is somewhat decreased, a decrease which is more evident as the secretion progresses and goes along with a change in pattern of distribution. The pyroninophilic substance is displaced to the apical edge of the cell, while the basal zone remains empty. It is thrown towards the glandular lumen which turns positive. The cells empty themselves of pyroninophilic substance and in the late secretory phase only light traces at the apex can be found. In the premenstrual phase the epithelium contains only these slight traces while the lumen is positive. The nucleoli during the whole secretory phase show a clear pyroninophilic reaction. The basal glands show only a weak and irregular reaction: many are negative and no change in the distribution during the course of the cycle is observed.

Alkaline phosphatase: The phosphatase in the glandular epithelium is seen during the entire proliferative phase. Of moderate intensity at first, it increases progressively and reaches a peak in advanced proliferation, occupying, then, the whole height of the cell. In the secretory phase a sharp decrease can be noticed; in the first days it is considerably reduced, being limited to a weak reaction in the apex of the cell, decreasing progressively and in the middle advanced stages of secretion no

reaction in the cells is seen. The basal glands show very little or no activity and there is no significant change. Except for the vessels the stroma is negative.

Postmenopausal endometrium: There is a great individual variation in the distribution of the substances investigated. Our attention has been directed principally to glandular dilatations observed in these endometria. The epithelium of such glands shows a very irregular distribution to the PAS reaction, moderately positive in general. It is a neutral mucopolysaccharide since it does not decrease normally by the diastase digestion or hyaluronidase.

The RNA is very scarce and of markedly irregular distribution. The alkaline phosphatase is moderately positive. What stands out most in the investigated substances is the marked irregularity of its distribution.

Simple proliferative adenomatous hyperplasia: The PAS reaction is moderately positive in the glandular epithelium, shows an analogous distribution to normal proliferative endometrium but is more irregular; digestion with saliva and hyaluronidase decreases this reaction only slightly. The stromal cells are negative. The RNA is intensely positive in the whole epithelial height of all the glands. The alkaline phosphatase is positive.

Cystic hyperplasia: The cystic dilations show a positive PAS reaction of irregular distribution sometimes occupying all the epithelial height, other times it is limited only to the apical part. Incubation with hyaluronidase scarcely modifies-if at all-the distribution of this reaction. Digestion with salivary diastase reduces somewhat the intensity of distribution.

The RNA also shows certain irregularities in its distribution. The pyroninophilic reaction is in general markedly positive in the whole epithelial height, although there are areas which show only traces or are absolutely negative. The alkaline phosphatase also shows irregularity in its distribution, but with greater constancy it is intensely positive in the epithelium of the dilations.

In spite of the cytochemical findings in endometrial tissue, attempts at looking for the same results in exfoliated cells obtained by endometrial aspiration have failed to demonstrate a correlation in the results, and no definite conclusion can be drawn.

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DISCUSSION

PIERRE HAOUR, Lyon, France:

I was very much interested in Galbis' work, which describes the cytochemical changes during the cycle and in physio-pathological conditions. Most of the substances investigated increase during the proliferative phase of the cycle, to be excreted into the glandular lumen.

I certainly do not have the cytochemical experience of Galbis, but I have also noticed the expulsion of positive PAS substances during the secretory phase of the cycle. I also found that this excretion corresponds to the apparition in the lumen of metachromatic material. Using toluidine blue, this reaction is evident throughout the secretory phase, violet blue vacuolated areas appearing in the glandular lumen, and this color contrasting with the pale coloration of the epithelium. At the end of the cycle, this reaction is also evident in the stroma in the intercellular net around the coiled arteries. It increases considerably in endometria with deciduiform reaction; the irregularity of distribution in the tubes was also noticed in cases of adenomatous hyperplasia. The metachromatic coloration could be used, we believe, as a very simple test to characterize the secretory endometrium.

I would like to ask Galbis what his opinion is concerning these metachromatic substances. The toluidine blue metachromatic reaction is specific for acid mucopolysaccharide; with the neutral the reaction is negative (1). Yet Galbis found that acid mucopolysaccharide is only located in the base of the cells and diminishes or disappears in the late secretory phase.

Concerning nuclei acid, I have no experience with RNA. The variation of D.N.A. content of glands and stromal cells has been very well established by histophotometry (2, 3); however, in several aspiration smears that we could control by histophotometry, we were not yet able to find any similar or significant variations.

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MARGARET E. LONG, New York, New York, U.S.A.:

The observations of Galbis on the cytochemical localization of substances in normal and abnormal human endometria agree in the main with findings reported by our laboratory (1, 2) and by others (3, 4). I am very interested indeed in his comments on the occurrence of acid mucopolysaccharides in the glandular cells. Following the PAS reaction, I have visualized only basal glycogen in cells of normal endometria. I wonder if Galbis would care to elaborate more specifically on these new observations? In our laboratory we strictly adhere to the McManus technique for the PAS method.

Galbis indicates that the stromal cells in the proliferative phase are positive for glycogen. Our experience has been that, in normal endometria, stromal glycogen appears only with the onset of the secretory portion of the cycle. Hyperplastic proliferative endometria of anovulatory patients previously treated with steroid compounds with progestational activity sometimes show an irregular stromal glycogen distribution.

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CLOSING REMARKS

MANUEL GALBIS, Valencia, Spain:

It is well known that the neutral and acid mucopolysaccharides are PAS positive, but only these latter give gamma metachromasia. We have no personal experience on the endometrial metachromasia, but the observations of Haour are all in accord with the general findings of McKay, Bozzo and others. They found that the metachromatic material in the endometrium is not influenced by the hyaluronidase. In our results we have found an intercellular PAS positive substance that irregularly diminishes by the action of the hyaluronidase in contrast with previous findings.

We have done the PAS reaction exactly as described by Hotchkiss. Using this technique we have obtained a positive reaction, not due to glycogen, in the apical and basal portions of the glandular cells. This reaction is decreased by the hyaluronidase. Only these last ones showed an irregular but definite decrease of the PAS positive substance. These have been our findings, although not in accord with others' experiences (see Figs. 1 and 2). The difference between our observations and the others has caused us some concern; we have checked our results and confirmed them.

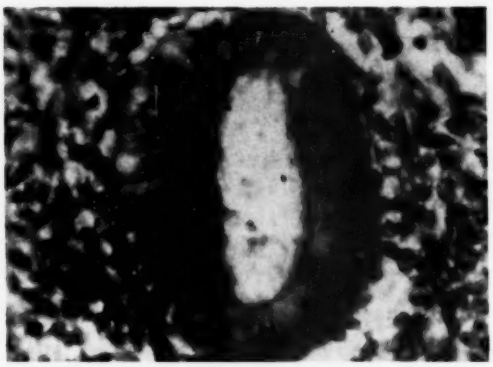

Fig. 1. PAS staining without incubation with hyaluronidase. A gland with PAS-positive substance in both apical and basal zones.

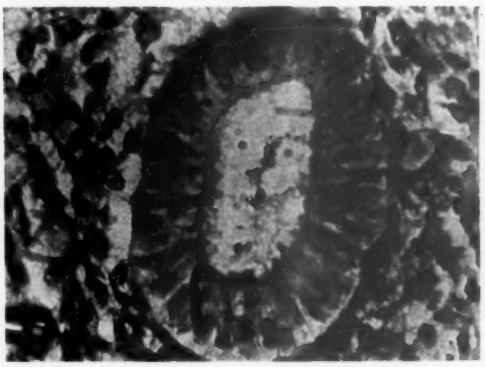

Fig. 2. PAS staining in the same endometrium after incubation with hyaluronidase. PAS reaction has decreased markedly, being limited to a small apical zone and irregular remainders in the other part of the gland.

In relation to the presence of glycogen in the stromal cells, we only found glycogen in the onset of the secretory phase.

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PHASEMICROSCOPY ON ENDOMETRIAL CELLS

HANS KLAUS ZINSER
Cologne, Germany

Among the cells desquamated from the endometrium, two main groups can be differentiated:

1. cells of the glandular lining and
2. cells of the stroma.

The cylindrical cells of the endometrium are characterized by their comparatively uniform shape. The basal part is slender and cone-shaped and sometimes it can be unusually long. The nucleus lies near the basal portion and is seen as a bright ball-shaped body in which a nucleolus is recognizable. In the phase contrast, the cytoplasm of fresh cells shows a dense granulation and sometimes small vacuoles. In single cases we deal with ciliated cells which come from the patches of ciliated epithelium in the uterine mucosa. In living material taken directly from the endometrium, you can see ciliary movement, often for hours. The cells of the uterine corpus are distinguished from the cervical glandular cells by their more slender and longer appearance. The structures of the cervical cells are more varied as a whole. They are larger, often cuboidal to fan-shaped or oval, according to the stage of the secretory function in which they are found. In contrast to the endometrial cells, the cytoplasm of the cervical cylindrical cells is vaculated or honey-comb-shaped, so that the nucleus is very difficult to see and lies at the base of the cell, as a structure with poor contrast. Many cellular forms of the uterine mucosa undergo, by being desquamated, considerable structural changes. Therefore, in the aspirated material numerous poorly preserved cells are found. Frequently many free nuclei of uniform shape and size are seen, the origin of which cannot be precisely determined.

The groups of cells originating from the stroma mostly lie in unions and are composed of fibrocytic, histiocytic or round-cell elements. Their nuclei are uniform in shape and the nucleolus of each is easily recognizable. Degenerative processes lead to conglomerations of nuclear clusters or to organization of cell complexes into net-like patterns. The criteria of the atypical endometrial cell, even in the phase contrast image, is often less significant than that of the squamous epithelial cell.

From a cytological point of view, differentiated adenocarcinomas of the uterus show much less qualitative deviation from the normal status than do squamous epithelium carcinomas.

On the other hand, in smears which can definitely be judged as containing atypical cylindrical cells, it cannot be determined whether we deal with an adenocarcinoma of the cervix or with one of the corpus uteri. Not only are cylindrical cells with nuclear atypias seen desquamating from less differentiated adenocarcinomas, but clusters of nuclei with extensively autolysed cytoplasm have been seen. The frequently occurring free nuclei often display all the known characteristics of malignancy. In cases like this, the structure of the endometrial cells is practically identical to the structure of dedifferentiated cells of a squamous carcinoma.

DISCUSSION

EDMUND SCHÜLLER, Vienna, Austria:

In the endometrial smear one can easily distinguish the highly cylindrical epithelial cells from the endocervix from the more cuboidal endometrial cells. Cilia are present in both cell forms. The identification of stroma cells, however, and their differentiation from epithelial cells of the endometrium pose considerable difficulties.

In highly differentiated adenocarcinomas the cytological diagnosis of cancer cannot be done with certainty because of the presence of homogeneous cellular elements. The hyperplastic endometrium frequently shows a considerable nuclear polymorphism in the smears which might lead to a false positive diagnosis. Phase contrast microscopy has practically no diagnostic value in direct endometrial smears. Useful specimens with a monocellular layer are not too easy to prepare. In aspirations

the cells are usually mechanically traumatized and one finds innumerable isolated nuclei in the smears and large clusters of tissue which are useless for cytological evaluation. The observation of the living endometrial cell is very stimulating for the cytologist and gives him valuable information when he compares the cytological picture with the histological section, even if the procedures which permit observation of the vital structures have very little practical diagnostic value, per se.

CLOSING REMARKS

HANS KLAUS ZINSER; Cologne, Germany: No comment.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

FLUORESCENCE MICROSCOPY ON ENDOMETRIAL CELLS

HORST SMOLKA
Kiel, Germany

Endometrial cell specimens prepared from aspirations, direct smears or scrapings show very suitable patterns for fluorescence microscopic examination. Unfixed as well as fixed slides can be used for examinations by secondary fluorescence. Histologic sections also exhibit excellent patterns. The cell structures in unfixed, fresh smears are very true to nature in the beginning, but may soon undergo alteration during the examination, owing to absorption of the highly intense irradiation. Apart from this, the coloration is often irregular. We prefer fixing the slides in ether-alcohol and can produce in a buffered aqueous solution of acridine orange, 1:10,000 with pH 6-7 (with subsequent dehydration and imbedding in Caedax/Bayer), impressive color differentiations with high brightness. The slides remain well-preserved. According to our experience, other fluorescent dyes such as herberine sulfate proved to be inferior to acridine orange. If acridine orange is used, the differentiation is based on different color shades, while, in the case of berberine sulfate, it depends only on different degrees of brightness.

We employ intense BV excitation with the combination of filters, BG 12 as primary filter and OG 4 and OG 5 (Messrs. Schott), the latter two being combined as secondary filters. The range of wave lengths serving for excitation lies between 5,500 and 8,000 A. The pictures obtained with the general and intense UV excitation were not as good as those obtained by intense BV excitation.

Acridine orange is particularly suitable for the demonstration of cells, as the structures appear in different colors ranging from green and yellow to orange and red. If little fluorescent dye is absorbed, the structures generally appear green; if more is absorbed, their appearance is red. In the case of fresh specimens, the devitalizing of the cells is accompanied by an increased absorption of fluorescent dye so that effete cells predominantly appear red (Strugger-Effect). There is no color which is specific for certain structures; however, it is possible to distinguish structural differences by the different colors. The tints always depend on the combination of filters, on the hydrogen ion concentration and on the quantity of fluorescent dye absorbed.

If the cytoplasm of singly lying endometrial cells is red-brown, the nuclei appear in yellowish or yellow-green shades with fine network structures. The nuclear membrane and the nucleoli show distinct yellow or orange color. Sex chromatin is also recognizable at the nuclear membrane. Dense fragments of epithelial tissue appear extremely bright and hardly show any details. The reticular structures of the stroma are excellently exhibited. Erythrocytes are not dyed, so smears containing abundant red blood cells are also suitable for examinations under the fluorescence microscope.

As to the value of this method for examinations of the normal endometrium, it can be said that in comparison to the diachrome staining procedures it does not seem to be of advantage for routine cytology at this time. For the purpose of scientific investigations, however, it is a very promising procedure.

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DISCUSSION

JEAN BERGER, Basel, Switzerland:

Like Smolka, we have also performed fluorescent microscopic investigations with fluorochromic acridine orange. There are certain conditions and rules in such fluorescent microscopic examinations for histology and for cytology as well. Although the fluoro-chromation is rather simple, uniform conditions would be requested in order to obtain uniform results.

The following points deserve special consideration:

a) The type of fluorescent dye, e. g., whether the dye is anodic or cathodic.
b) The concentration of the solution.
c) The pH.
d) The duration of application.
e) The temperature.
f) The wave length of the light source used.

If a series of increasing pH of acridine solution is used, one will find the transitional change from red to green sooner or later, depending on the cell composition (Smolka). Atypical cells will fluoresce from a yellowish to an orange color at low pH. Whether we deal with a shift of the isoelectric point to the acid side or with a decomposition of the cells allowing the dye to absorb faster is not well-known. In a series of examinations, which we will report in the very near future, we found that the more atypical the cells are, the lower the pH at which they will fluoresce yellowish-orange. This orange color is duller and we have never found a shiny bright orange fluorescence, not even at higher pH.

The results show that fluorescent microscopy is a promising method. It is also felt that a great deal of new knowledge for cytodiagnosis (where fluorescopy offers beautiful structural differentiations) will spring from this method.

CLOSING REMARKS

HORST SMOLKA, Kiel, Germany:

I fully agree with the technical supplements made by Berger. These also confirm the important fact that in investigations with fluorescence microscopy, it is necessary to state the exact technical conditions. Only in this way will it be possible to obtain reproducible and comparable results on biological material.

DIFFERENTIATION OF ENDOCERVICAL AND ENDOMETRIAL CELLS

HORST SMOLKA
Kiel, Germany

The differentiation of endocervical and endometrial cells often meets with difficulties. It may be easy in typical cases; however, there are cellular pictures in which, even if long experience is available, a definite morphologic distinction is hardly possible. Some characteristics are as follows:

Endocervical cells: There are two types of cells which can be distinctly differentiated: the secretory and the ciliated cells. In endocervical smears the secretory cells are found in abundance, whereas the ciliated cells almost always occur in considerably smaller numbers. The secretory cells are short and goblet-shaped or they may also be slim, occasionally tube-shaped. The cytoplasm (stained according to Papanicolaou) is relatively light, mostly yellowish during secretory activity. It may be homogeneous or show a fine vacuolization. The nucleus is located basally and mostly exhibits a finely granular structure. In fragments of epithelial tissue the arrangement of the cells looks honey-comb-like if seen vertically. The nuclei then mostly appear to lie eccentrically. It is relatively easy to distinguish the ciliated from the secretory cells, as the cytoplasm of the ciliated cells stains always cyanophilic and consequently often has a dark appearance. Frequently there are one or more vacuoles near the centrally located nucleus. The light-red cuticular membrane, densely ciliated, represents the distal border of the cell. The nuclei of ciliated cells often appear darker than those of secretory cells. This may be explained by the fact that they undergo autolysis less quickly. Multinucleation is frequent in ciliated cells. Both types generally show one or two nucleoli in the nucleus, and in many instances the sex chromatin is very distinctly seen at the nuclear membrane.

Endometrial cells: The epithelial cells of the surface and the glands have to be differentiated from the cells of the stroma. The columnar or cuboidal epithelium also shows secretory and ciliated cells. In the smear, however, these cells can rarely be distinguished from each other with any degree of

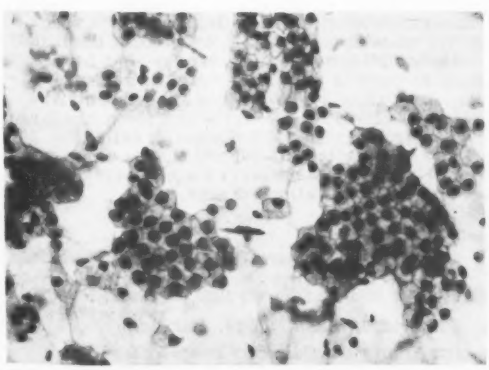

Fig. 1. Endocervical columnar cells in small fragments of epithelial tissue seen vertically.

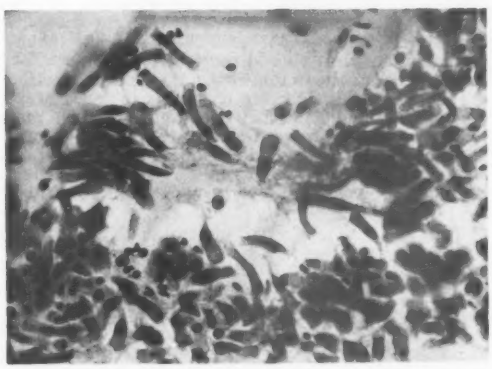

Fig. 2. Endocervical secretory cells.

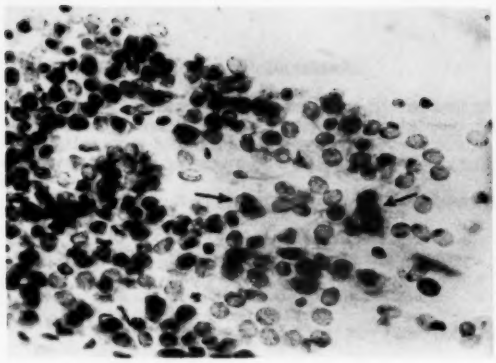

Fig. 3. Free nuclei of endocervical secretory cells. The darker cells indicated by arrows are ciliated cells.

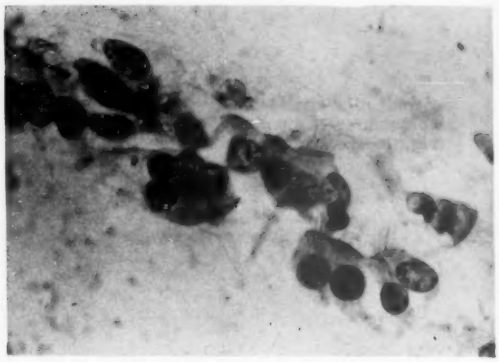

Fig. 4. Endocervical ciliated cells.

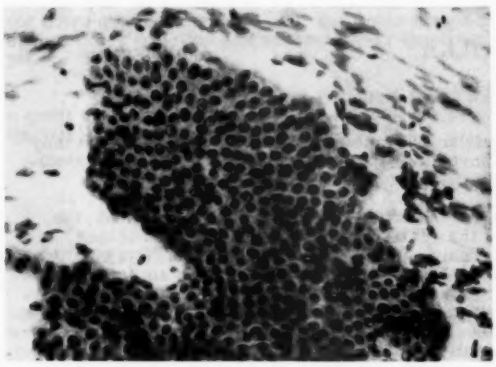

Fig. 5. Fragment of endometrial epithelium. The cells are seen vertically.

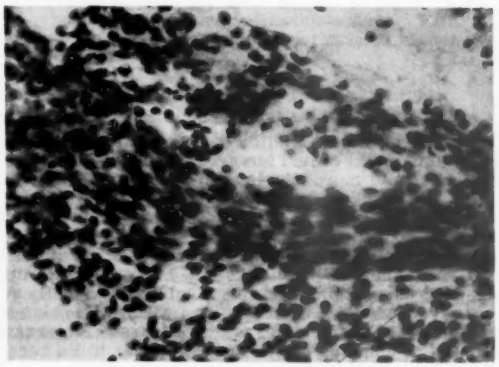

Fig. 6. Endometrial stromal cells.

certainty. This may be partly due to the fact that these cells have less ample cytoplasm than the endocervical cells. There are frequently no discernible cell borders. Apart from this the cilia of the endometrial cells are extremely delicate and are very easily lost. The stroma contains syncytial, reticular cells with large, light and small, dark nuclei, as well as free cells such as histiocytes, monocytes, lymphocytes, etc. All these stromal cells show relatively polymorphic patterns. When occurring in clusters or fragments of tissue they lie in a very entangled arrangement and do not exhibit the regular tissue formation of the cuboidal or columnar epithelium.

Differentiation: Though there may be certain cyclic variations, the endocervical cells (Fig. 1) are usually larger and more robust than the epithelial endometrial cells (Fig. 5). Compared with endometrial cells, the endocervical cells generally have relatively more ample cytoplasm and more distinct cell borders. When viewed vertically, the endocervical cells exhibit a well-defined honey-comb form. This form is rarely so marked in endometrial cells. The appearance of the tissue structure is denser and sometimes a little more irregular in endometrial cells. In addition to that, epithelial endometrial cells have smaller and more coarsely granular, mostly darker nuclei (Fig. 5). However, the nucleoli and the sex chromatin are well recognizable in spite of this. The variable stromal endometrial cells (Fig. 6) are only rarely confused with endocervical cells because of the ordinarily well-defined shapes of the cytoplasm of the latter. Even when the cytoplasm undergoes autolysis the free nuclei of the endocervical cells usually appear regularly round or oval, although there are frequently variations in size.

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DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

I am in hearty agreement with the paper of Smolka. To us, the differentiation of endometrial from endocervical cells never presents any great difficulties regardless of the type of smear. I want to emphasize that it is even possible to differentiate naked nuclei of endocervical cells from those of endometrial cells since the latter are smaller, darker and often more slender.

WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.:

I agree in the main with Smolka. It is usually easy to differentiate endocervical from endometrial cells utilizing the criteria Smolka outlines. Endocervical cells are larger with better preservation of the cytoplasm. The source of the smear may be helpful at times in questionable cases. Occasionally, histiocytes may be confused with either endocervical or endometrial cells. It is important to recall that so-called endocervical cells of columnar type may occur on the cervical portio, especially in cases of cervical "erosion" and for that reason the findings of "endocervical" cells does not necessarily mean that the cervical canal has been cytologically sampled.

HANNAH PETERS, Bombay, India:

Smolka is quite right to emphasize that the differentiation of endocervical and endometrial cells is often not possible with certainty. However, as endometrial cells do not normally appear in vaginal or cervical smears except during menstruation, it is important to be able to identify them, as their presence even in an otherwise normal smear might signify an unusual or pathological condition.

GUILLERMO TERZANO, Buenos Aires, Argentina:

I agree wholeheartedly with Smolka's presentation. In smears prepared and stained according to Papanicolaou's technique, it is difficult to be certain sometimes where a cell is derived from, when the diagnosis is to be made by the morphological aspect of a single (cuboid or columnar) cell. Fortunately, in practice, this is not very often necessary. A thorough study of the smear usually gives enough information for determining the cell origin because endocervical cells and endometrial cells seldom appear isolated, but in groups or clusters, and the source from which the specimen has been obtained is known.

LUBA A. TURNBULL, Montreal, Canada:

I do agree with Smolka about the characteristics of the normal endometrial and endocervical gland cells, but I must say that it is more difficult to differentiate the endometrial cells in smears than the endocervical cells.

There are a few factors that make it difficult for the cytologist to be accurate in recognizing the endometrial cells:

1. The endometrial cell is comparatively smaller in its diameter than the other epithelial cells and sometimes not even larger than a leukocyte. It is round or oval in form and a single cell could easily be lost between the cells in vaginal secretion.

2. In smears endometrial cells appear mostly in clusters.

3. The endometrial clusters often include histiocytes, leukocytes, bacteria and mucus, which makes it difficult to recognize and to study the cell.

Endocervical cells are found very often in smears from the cervix taken with a wooden spatula. They lie in groups or singly and the cilia are not always visible. These endocervical cells have elongated cytoplasm and round nuclei which can be seen close to either end of the cell.

Cells which have been applied carefully on the slides by trained hands are not difficult to recognize.

CLOSING REMARKS

HORST SMOLKA, Kiel, Germany:

I am glad that the discussants agree with me and I am grateful for the supplementary experiences reported by them.

In findings of endometrial cells I would suggest that it should be stated more often, whether these cells originate from the epithelium or from the stroma. It is striking that this differentiation is only rarely made in cytologic publications, though it would be possible in many cases to give a more exact definition of the origin of the cells.

HISTIOCYTES AND ENDOMETRIAL CYTOLOGY

HERBERT E. NIEBURGS
New York, New York, U.S.A.

Endometrial smears obtained by aspiration from the cervical os or directly from the uterine cavity contain a variety of cells. The understanding of the different cell types and their significance in their relationship to physiologic and pathologic changes in the endometrium, are important. In smears, the endometrial cells appear either in a sheet-like formation as the round type with a small rim of cytoplasm and are of glandular origin, or they appear as elongated cells of stromal derivation. The variation in the size of the cytoplasm is of little importance for the diagnosis of malignancy. The variation in nuclear size, though of some significance, is not the determining factor for the interpretation of the cell atypia. The nuclei of endometrial cells of glandular origin have evenly granular chromatin and vary between 6 and 10 micra. The larger size is usually found during the secretory phase. Adenomatous hyperplasia of the endometrium is usually associated with a larger nuclei, approaching 10 micra or above, though usually below 15 micra. With polypoid hyperplasia of the endometrium, cells are found with nuclei between 8 and 10 micra. In adenocarcinoma of the endometrium, nuclear size may vary between 8 and 25 micra, with coarse, irregular nuclear particles and large nucleoli. Endocervical cells, when present, or endometrial cells from areas of squamous metaplasia are usually recognized with little difficulty.

If the morphology of the different cell types in endometrial smears is well understood, the presence of histiocytes does not contribute significant difficulties in the differential diagnosis of cells. Endometrial histiocytes are usually present in increased numbers towards the end of menstruation and during the early proliferative phase. They are characterized by nuclear size varying from 4.6 to about 8 micra, by their distinctive nuclear shape and by chromatin appearance. Although there may be an overlapping between the nuclear size of histiocytes and of malignant cells, the different shape of the structure of the nuclei in histiocytes is sufficiently distinctive for their adequate interpretation. Often transitional changes between normal endometrial cells and histiocytes are seen. Transitional stages may also be observed between stromal and epithelial endometrial cells.

Although the transitional forms, per se, may be difficult to evaluate, the following points facilitate the interpretation of the transitional cells:

1. The characteristic histiocytes found in the periphery of the endometrial cell clusters, with their usually evenly distributed nuclear material.
2. The decreased staining reaction which gives these nuclei a pale appearance.
3. The frequent coffee bean-shaped appearance.

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DISCUSSION

OLAF T. MESSELT, Oslo, Norway:

I agree with Nieburgs in his statements. I only would like to emphasize that if one is not too familiar with the morphology of exfoliated cell types of the endometrium, the cells from the endometrial stroma which may be exfoliated in the postmenstrual stages and which have rounded hyperchromatic

nuclei may present difficulties. The same is true of endometrial cells in early proliferative stages, which, according to Papanicolaou, differentiate into phagocytes. The nuclei in these cells can often be irregular in shape, the chromatin granulated and the cytoplasm sometimes vacuolated; these findings may lead an inexperienced interpreter astray.

CLOSING REMARKS

HERBERT E. NIEBURGS, New York, New York, U.S.A.: No comments.

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CYTOMETRY ON NORMAL AND ABNORMAL ENDOMETRIAL CELLS

HANNS-WERNER BOSCHANN
West-Berlin, Germany

I. Cytometry of the Normal Cycle

Figure 1 shows the results of 200 ocular micrometer measurements taken on each day of the menstrual cycle. The arithmetical mean of the relative surface area of the nuclei (that is, the product of the longitudinal and the oblique diameter of the nuclei) is given.

The two upper lines show the behavior of the stroma cell nuclei (fine solid line) and the behavior of the stroma cells themselves (fine dotted line). In accordance with the figure, the stroma cell surface and the stroma cell nuclear surface increase during the proliferation phase until approximately the 13th day of the cycle: the cellular surface from 45 μ^2 to 72 μ^2 and the nuclear surface from 35 μ^2 to 52 μ^2. After that time a decrease (apparently post-ovulatory) of both values occurs to almost the initial figures: cellular surface decreases to 52 μ^2, nuclear surface to 38 μ^2. Between the 18th and 20th day of the cycle, a second increase of the cellular surface can be observed. This increase remains until menstruation and reaches its preovulatory peak. The nuclear surface shows an increase up to preovulatory size until the 24th day of the cycle. This is followed by a decrease to post-ovulatory values until the 28th day.

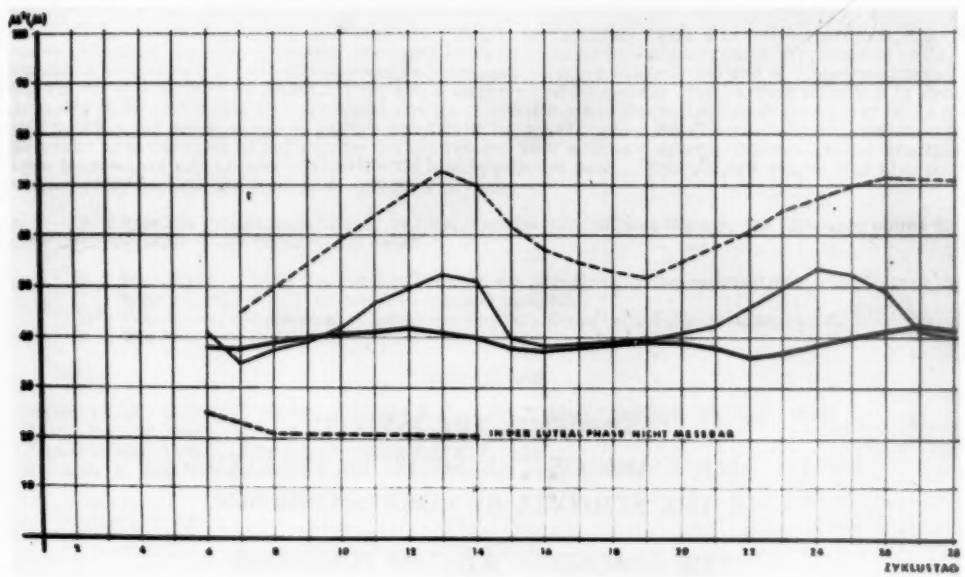

Fig. 1

The nuclear surface of the glandular cell has been shown by a heavy solid line. The values range around 40 μ^2. Also, a preovulatory increase in size and a post-ovulatory decrease in size seem to be present here. However, this is not statistically significant.

The height of the epithelium (in micra) is shown by the heavy dotted line. In this instance no increase in values could be observed. As a consequence of the loss of structures of the glandular cells during the secretion phase, measurements in this half of the cycle could not be made.

These changes during the normal cycle are characteristic but they are not statistically significant. However, they largely agree with the results found with histological (endometrial) preparations.

II. Cytometry of Normal and Abnormal Endometrial Cells

Figure 2 shows the variation curves of all of the nuclei in the normal cycle, in menopause, in glandular hyperplasia and in carcinoma.

The averages were as follows:

Normal Cycle:	
Stroma Nuclei	$46.2 \pm 7.8\,\mu^2$
Glandular Cell Nuclei	$40.7 \pm 5.9\,\mu^2$
Menopause:	
Stroma Nuclei	$36.8 \pm 6.1\,\mu^2$
Glandular Cell Nuclei	$36.8 \pm 7.8\,\mu^2$
Glandular Hyperplasia	$51.5 \pm 13.3\,\mu^2$ (7 cases measured)
Adenocarcinoma	$87.0 \pm 30.9\,\mu^2$ (11 cases measured)

The conclusion from this is that groups of all stroma and epithelial nuclei in the normal cycle and during menopause is different from the cancer group. This is statistically very significant (p is less than 0.001). The glandular hyperplasia group takes on an intermediate position. It still differs from the group of adenocarcinomas by a statistical significance (p is less than 0.01).

Compared with the group of cells from the normal cycle, menopausal cells (Fig. 2) show a shift to the left which easily can be seen in the above table. The normal variations of the endometrium (cyclic and menopausal) show only a small range, which is shown by the peak in the curve.

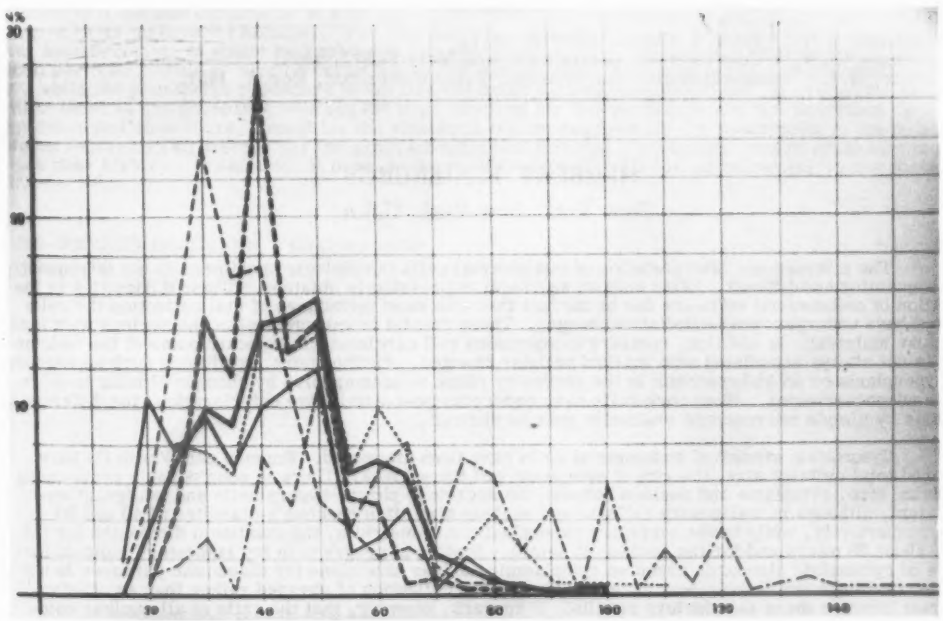

Fig. 2

With glandular hyperplasia the flat extensive curve indicates a marked variation in nuclear size. The same can also be concluded from the average mean deviation ($\pm 13.3\mu^2$ compared to $\pm 7.8\mu^2$ and $\pm 5.9\mu^2$ in normal cycle and to $6.1\mu^2$ and $7.8\mu^2$ in menopause).

The carcinoma group shows by far the maximum deviation. This is shown by the large mean deviation of $\pm 30\mu^2$ and also from the irregular multi-peaked curve. The largest nucleus we measured in the group had a surface $820\mu^2$, the smallest $16\mu^2$. Thus, for corporal carcinoma not only the absolute nuclear size of $87\mu^2$ is characteristic, (as compared to (a) the normal cycle with a nuclear size of $45.2\mu^2$ and $40.7\mu^2$, (b) menopause with a size of $36.8\mu^2$ and (c) glandular hyperplasia with a size of $51.1\mu^2$) but the large mean deviation also is characteristic.

This distribution curve agrees mainly with that found by Cramer in vaginal and cervical cytology. Cytometrically the sarcoma is similar to carcinoma. On the other hand, the normal endometrial variations can well be differentiated. Benign uterine polyps are cytometrically similar to the glandular hyperplasia.

Summary

The entire adenocarcinoma group differs statistically very significantly (p is less than 0.001) from the entire group of all stroma and epithelial cell nuclei in normal cycles and in menopause. It also differs significantly (p is less than 0.01) from the entire group of glandular hyperplasia.

The menopausal cells show a shift to the left as compared to the group in the normal cycle (i.e., to the smaller values).

For the corporal carcinoma we found the following characteristics:

1. Increased absolute nuclear size
2. Large mean deviation from the average

Thus, in doubtful cases, cytometry can be used quite well to differentiate between normal endometrial variations and adenocarcinoma and sarcoma.

*The more than 20,000 measurements have been made together with Dr. H.J. Zörner.

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HERBERT E. NIEBURGS
New York, New York, U.S.A.

The microscopic interpretation of endometrial cells in cytologic specimens is not infrequently time-consuming and difficult. Often only an equivocal impression is obtained. These difficulties in the evaluation of endometrial cells are due to the fact that with most techniques of cell collection the cells obtained have undergone post-exfoliative changes. These consist in enlargement of the nucleus with loss of nuclear material. In addition, contrary to squamous cell carcinoma, adenocarcinoma of the endometrium is not always associated with marked cellular changes. Furthermore, conditions such as adenomatous hyperplasia or an endometrium in the secretory phase is accompanied by changes similar to those seen in adenocarcinoma. When such cells have undergone post-exfoliative deterioration, the differential diagnosis by simple microscopic evaluation may be difficult.

Cytometric studies of endometrial cells have been attempted. Hopman and Werch (1) have measured total cellular size, the rim of cytoplasm and the nucleus. There is considerable overlapping of cellular size, cytoplasm and nucleus between the secretory phase, hyperplastic and malignant endometrium, although in malignancy cellular and nuclear size often reaches a diameter of 40 and 50 micra respectively, while in the secretory hyperplastic endometrium, the maximum dimension for the cell is about 20 micra and for the nucleus 15 micra. Studies in progress in my laboratory indicate that the use of cytometric standards based on cytoplasmic nuclear size alone for diagnostic purposes is not reliable. Perhaps the development of techniques for the collection of abraded rather than exfoliative cells may produce more satisfactory results. It appears, however, that the ratio of all nuclear components to the size of the nucleus is a determining factor in the differential diagnosis between non-malignant and malignant lesions of the endometrium. A report on the techniques used and standards applied will follow shortly.

Boschann's calculation of micrometer measurements of endometrial cells differs slightly from ours. Whereas Boschann multiples the longitudinal by the oblique diameter of the nuclei, we have recorded the mean nuclear diameter of two measurements. It appears that the results essentially agree with ours. It has to be pointed out, however, that contrary to the statistically significant difference between endometrial nuclear size during the normal cycle, menopause, glandular hyperplasia and adenocarcinoma, there is a certain amount of overlapping between different cell types which limits the practical diagnostic value of nuclear measurements. In our material overlapping of the mean linear nuclear measurements occurred in the presence of cells from polypoid hyperplasia, secretory phase and particularly, placental tissue when the diameter was below 14.5 micra. We have therefore come to the conclusion that the evaluation of the nuclear particles in addition and in relation to the nuclear diameter is essential and of greater significance than the nuclear diameter alone.

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DISCUSSION

GEORGE L. WIED, Chicago, Illinois, U.S.A.:

Caspersson and Fredriksson developed an ingeniously simple, inexpensive and highly accurate cytometer (Hereditas 49:201, 1953) which should be used much more by those of us who are concerned with determinations of cellular, nuclear and nucleolar sizes. This is by no means a criticism of the very interesting and undoubtedly time consuming work of both authors, Dr. Boschann and Dr. Nieburgs.

CLOSING REMARKS

HERBERT E. NIEBURGS:

The microplanimeter developed by Caspersson, Fredriksson and Thorsson is a very useful instrument for linear cellular measurements. As Wied points out in his discussion and in accordance with the author's own description of the procedure, the instrument was used for measurement of cytoplasm, nucleus and nucleolus. This is not a time consuming procedure even if carried out by other means.

From my own studies on the measurement of gastric and endometrial cells, it appears, however, that no satisfactory degree of diagnostic accuracy could be maintained by limiting the measurements solely to cellular, nuclear and nucleolar sizes. I found it necessary to measure all nuclear particles and to elaborate well-defined criteria as to what type of particles are significant for the interpretation of the cell in regard to its malignant character. For this purpose, a planimeter was developed which permits the linear measurements of single cell particles and which, in addition, records the total linear diameter of all cell particles. This is carried out on a polaroid photomicrograph which is taken at a magnification of 2000, while the planimeter procedure is carried out under further magnifications. It is doubtful as to whether there is any difference between the time required for this procedure and that described by Caspersson and co-authors, except for the additional minute required for the preparation of the polaroid photomicrograph. Our method has the great advantage of securing a permanent record which is attached to each case history. In addition, it permits the identification of cells for measurement by our technical staff.

HANNS-WERNER BOSCHANN: No Comments.

CYTOLOGY OF ENDOMETRITIS

HANNS-WERNER BOSCHANN
West-Berlin, Germany

The penetration of the stroma by inflammatory cells is characteristic for unspecific endometritis. Besides cellular detritus we find plentiful leukocytes, erythrocytes, lymphocytes and histiocytes according to the stage of inflammation.

In endometritis post-abortum, the stroma (penetrated by inflammation) is transformed into a decidua-like tissue, i.e., each stroma cell has a thicker rim of cytoplasm and a loose, lighter staining nucleus. Moreover, we find chorionic elements, particularly if pieces of placental tissue have been left in situ. These elements appear as simple chorionic migration cells with a large, chromatin-rich nucleus, or in other instances as multi-nucleated giant cells with eosinophilic cytoplasm and a large amount of irregularly shaped chromatin. The chorionic cells allow differential diagnosis for ectopic pregnancy (which cannot be done by mere vaginal cytology).

In endometritis post-abortum, one usually finds many erythrocytes. In endometritis tuberculosa frequently the only finding is that of an unspecific endometritis. Sometimes, however, we see multi-nucleated giant cells, the peripheral arrangement of their nuclei resembling that of Langhans' giant cells.

Multi-nucleated giant cells are also found in smears of foreign body endometritis, as well as in carcinoma and sarcoma.

Differential diagnosis for malignant changes:

Although nuclei in endometritis usually show an increased content of chromatin (they "look more active"), they do not show the extreme nuclear polymorphy of carcinoma, with its hyperchromasia, indentation of the nuclear membrane, monstrous nuclear shapes and great variety in size.

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DISCUSSION

JEAN A. de BRUX, Paris, France:

I agree with Boschann on the description of the cells exfoliated from non-specific endometritis.

However, I do not recall having met with cells of decidual type, nor many chorionic elements, in the post-abortum endometritis. I believe that it is necessary, first of all, to agree on the meaning of the term "post-abortum endometritis." In my opinion, this generally involves inflammation of an endometrium on which there persist, for several days or even for several weeks following the accident, either fragments of decidua or chorion-placental villosities. The cells of these fragments are usually necrotic, in the midst of exudates of fibrin and of altered polynuclears, and consequently are no longer recognizable, even by aspiration. If they persist in the form of strips, they are encased by a proliferative endometrium, and hence no longer exfoliate.

Nevertheless, in a certain number of cases the diagnosis, although difficult, may be made: in particular, when there appear stromal and epithelial modifications secondary to the presence of chorionic

vestiges. These lesions were discussed for the first time by Arias-Stella, and later her study was reopened by us with Dr. Vaissade. The cells are "blown up," with the cytoplasm containing a very large vacuole and are P. A. S. and muci-carmin positive. The large, irregular and chromatic nucleus undergoes a beginning of pyknosis. It is situated in the center or at the apical part of the cell. The stroma cells are very voluminous and chromatic and give such an impression of "activity" that they may easily be confused with sarcomatous cells.

All of these factors render a precise diagnosis difficult, and often only anamnesis and the patient's age will suggest the causal origin of the cytological images.

Hence, I do not share the euphoria of those cytologists who claim that the cytological diagnosis of the inflammatory or tumoral endometrial lesions is easily reached.

As to the tuberculous endometritis, I raised this hypothesis in one case, based on the findings of a multinucleated cell of Langhans' type. The biopsy confirmed the diagnosis. But since then I have encountered no similar cases.

CLOSING REMARKS

HANNS-WERNER BOSCHANN:

I agree with de Brux that endometrial cytology allows no euphoria. The criteria described according to our findings are not always present, but if they occur, they are very helpful for the practical diagnosis. As in cytology on squamous epithelia, the last decision depends on the biopsy.

CYTOLOGY OF ENDOMETRITIS TUBERCULOSA

GUILLERMO TERZANO
Buenos Aires, Argentina

The endometrial smears of 15 women with histologically-proven endometritis tuberculosa have been studied: 5 follicular productive, 3 caseous and 7 clinically cured cases (pathological reports). The material was obtained by endometrial aspirations.

In seven cured cases the smears did not show any abnormal cytology apart from a few isolated Class II cells with no special features.

The cases with five follicular productive endometritis tuberculosa were found to contain several striking factors: intense desquamation of endometrial cells, singly, in clusters or in sheets, showing anisokaryosis and large irregular nuclei rich in chromatin and with a heavy nuclear membrane. Some of the cells were as large as the cells derived from the superficial lining of the endometrial cavity, while others were smaller, like the stromal cells. Many of the cells had signs of degenerative changes such as pale nuclei, wrinkled nuclei, etc. Stripped nuclei or free nuclei were remarkably numerous. In one case, a large multinucleated giant cell having the morphological aspects of the Langhans-like cell, as described in the literature was present. In two other cases of genital tuberculosis, giant multinucleated cells were present in the vaginal smears, but the final diagnosis in these two cases was "tubal tuberculosis" with no endometrial lesion.

In the three cases of caseous endometritis tuberculosa, clusters of endometrial cells showing anisocytosis and anisokaryosis were the principal characteristics of the smears. Histiocytes, poly-

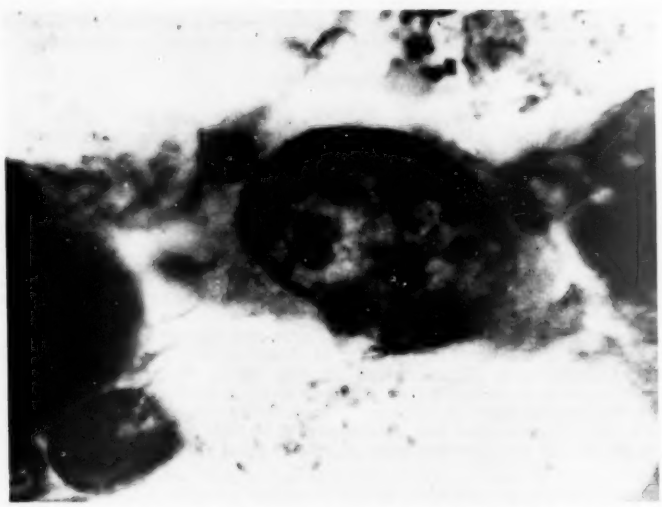

Fig. 1. Endometritis tuberculosa, endometrial cells (G. Terzano)

Fig. 2. Endometritis tuberculosa, stroma cells (G. Terzano)

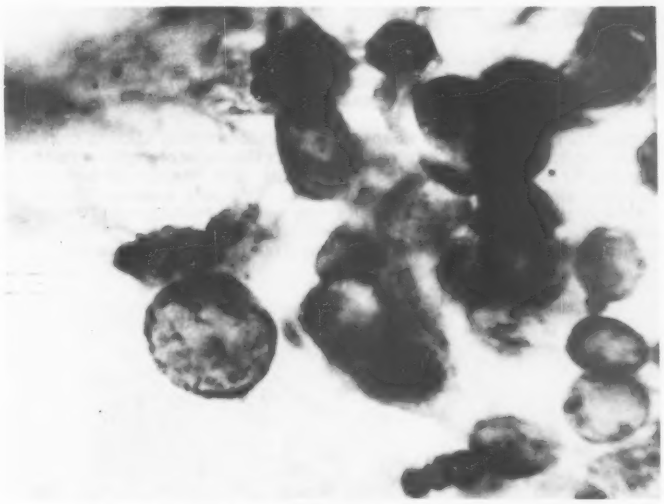

Fig. 3. Endometritis tuberculosa, free nuclei (G. Terzano)

morphonuclear leukocytes, lymphocytes and erythrocytes were present both in quality and in quantity as in any chronic inflammatory reaction. They cannot be considered as a dependable point of reference for the diagnosis.

When the presence of tuberculosis was suspected or previously known, it was not surprising to have found these abnormal cells, but in those cases in which the smears were screened for cancer diagnosis (patients complaining of bloody discharge, watery discharge, etc.), the cells were classified as Class III (suspicious). I might mention also that we have vaginal smears of 21 women with clinically proven genital tuberculosis on file but without pathological reports because no curettage was performed.

I think that the tuberculous nature of an endometrial disease can be suspected by endometrial cytology. The rather rare presence of giant Langhans-like cells may help in the diagnosis. Nevertheless, the endometrial aspiration smear cannot be considered as a reliable diagnostic procedure for the detection of endometrial tuberculosis.

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DISCUSSION

JORGE CAMPOS R. de C., Lima, Peru:

Endometritis tuberculosa and even tuberculous cervicitis are not rare in our material, therefore, we have had the opportunity to study the cervical smears in some patients with these conditions. We could not find in any case a characteristic cytology which allows us to make the diagnosis of tuberculous infection, but in some cases endometrial or endocervical smears may suggest that condition.

The large multinucleated giant cells which Terzano describes are not characteristic; we found them in tuberculous endometritis as well as in other infectious or inflammatory diseases of the endometrium and cervix such as trichomoniasis, post-resection of the cervix, etc.

Generally, we found many leukocytes and cellular debris especially in those exudative cases in which there is a predominance of caseation. In these cases cytology can help in the diagnosis.

In short, I agree with Terzano's conclusion.

F. A. IKLÉ, St. Gallen, Switzerland:

Cytology offers very little for detecting or controlling tuberculosis of the uterus or its appendages. This is not surprising when we remember that even by uterine curettage, endometrial tuberculosis is missed in 20 to 25 percent of the cases (Jeffcoate).

When endometrial tuberculosis is suspected, a premenstrual uterine curettage should be done. The curettings are used for histological examination and for the culture and/or guinea pig inoculation tests. If no curettage can be done, the collected blood of the first menstrual day can be used for culture or inoculation. Aspiration smears are not at all accurate. Our only smear strongly suspicious for tuberculosis among 9500 routine cases was scraped from an ugly looking cervix with bright bleeding and red papillary "erosion" (Fig. 1). Note the epitheloid cells and the multinucleated giant cells of the Langhans-type. Cervical tuberculosis was confirmed by histological examination.

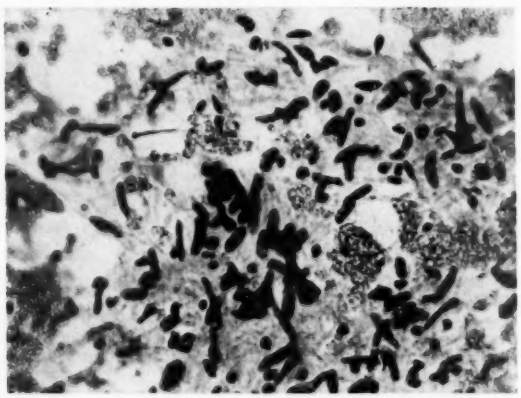

Fig. 1. Cervical Smear - Tuberculosis of the Cervix (F. A. Iklé)

JULIETA C. DE LAGUNA, Mexico, D.F., Mexico:

We have studied vaginal and cervical smears in 13 cases of tuberculosis of the endometrium. Two of them simultaneously had carcinoma and were accordingly diagnosed as positive: One was adenocarcinoma of the endometrium and the other was carcinoma in situ of the uterine cervix (cytological detection). The remaining 11 cases were diagnosed as follows:

Inflammatory reaction. No endometrial exfoliation.
Negative I — 5 cases

Endometrial cells with Class II alterations.
Negative II — 2 cases

Frank atypias in columnar epithelium. No malignant changes.
Negative III — 3 cases

Large amount of blood. Endometrial Class III doubtful of malignancy.
Doubtful III — 1 case

As can be seen, the direct diagnosis of tuberculosis has never been made in our laboratory, but we think it worthwhile to emphasize the point that almost all of these cases were classified as inflammatory conditions, and they did not become confused with tumoral pictures.

CLOSING REMARKS

GUILLERMO TERZANO: No comments.

CYTOLOGY OF ENDOMETRIAL POLYPS

JEAN A. de BRUX and JACQUELINE FROMENT-DUPRÉ
Paris, France

Before starting, it is necessary to define the term "endometrial polyp." It is an intra-uterine, permanent and stabilized formation without any tendency to be spontaneously eliminated or to become malignant.

This definition excludes from the subject:

1) the endometrial polypoid hyperplasia.
2) the endometrial carcinoma.

But two functions must be included which have different pathological and cytological expressions:

1) the glandulocystic and fibrous polyps.
2) the submucous fibroids.

A. The Glandulocystic and Fibrous Polyps

These formations represent only the terminal state of a functional and hyperplastic endometrial proliferation. They are progressively formed in the following two manners:

a) the persistence and the growth of the endometrial vessels which "anchor" the proliferation onto the uterine wall (Figs. 1a, 1b).
b) the fibroblastic organization of the exudates and hemorrhages which, for a rather long time each month, modify the endometrial stroma (Fig. 2). This latter shows a very incomplete and superficial desquamation.

One can say that most of these glandulocystic and fibrous endometrial proliferations are "titularized" and reorganized hyperplasias. The "true" endometrial polyp might be considered as foreign

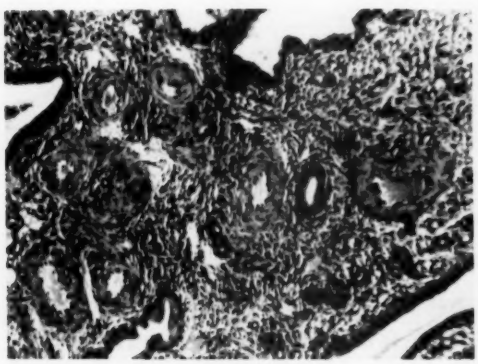

Fig. 1a

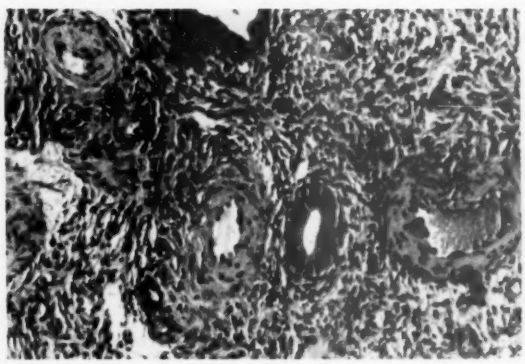

Fig. 1b

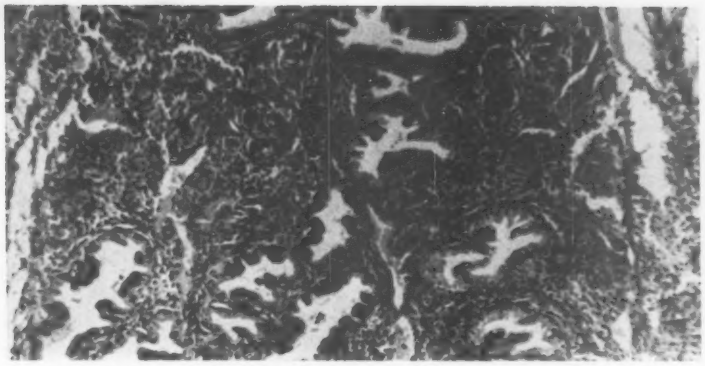

Fig. 2

tissue coming from the uterine mucosa on which it is attached only by a pedicle, more or less large and vascularized. Thus, the polyp no longer participates in the physiological life of the endometrium and remains insensitive to the periodical hormonal stimuli. Before this stage, however, it still undergoes these stimulations to a more or less marked extent. The zones not yet sclerosed are still sensitive to hormonal stimuli. The progesterone response is the first to disappear; the estrogenic action is of longer duration. The glandular tubules closest to the functioning capillaries undergo the hormonal actions for the longest periods. Those nearest the sclerosed pedicle, or in the periphery, are the first to lose their sensitivity; some become cystic, others atrophic. The stroma widens and becomes hyperplastic and sclerosed. The polyp so constituted has no tendency to become a cancer, in contrast to the adenomatous hyperplasia. When a cancer is developing in the uterus, the growth starts from the neighboring endometrium and not from the polyp itself. These facts explain the relative poverty of the exfoliative cytology of the glandulocystic and fibrous endometrial polyps.

We can see only a few clumps of retracted and subatropic endometrial cells. The nuclei of these cells are dense, with irregular borders and poor chromatous reticulums. A perinuclear halo appears in the cytoplasm, produced by the nuclear retraction (Fig. 3). Sometimes the clumps are small with only 5 to 10 cells. The cyanophilic cytoplasm is only a tight ring. The nuclei are piled up. The chromatin is dark and dense (Figs. 4, 5). Often the inflammation results in the exfoliation of tissue fragments. Moreover, many inflammatory cells are mixed with these cells. Polymorphonuclear leukocytes, histiocytes, nuclear debris, free nuclei, dark cyanotic chromatin, (Fig. 6) and vacuolized cells give the smear a very "dirty" aspect.

Hence, the differential diagnosis of endometrial carcinoma is very often difficult. However, the adhesion of the cell clumps plus the cytological context of the vaginal smear, permit a distinction between the two lesions. In some instances, the difficulty is even greater because on the same smear the atrophic and hypertrophic cells from a severe inflammation may be associated with these malignant cells. Here the diagnosis of carcinoma is possible. In any case, in the presence of difficulties in cytologically determining the benignity or malignancy of an endometrial lesion, other instrumental explorations, and especially the biopsy, must be performed.

B. The Submucous Fibroids

The histological nature of the submucous fibroids, resembling in appearance the endometrial polyp in the uterine cavity, is most often a pathological discovery. However, twice we have been able to

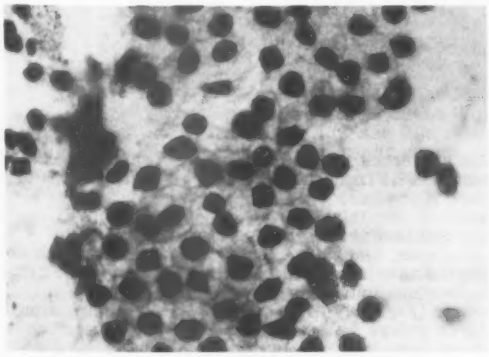

Fig. 3

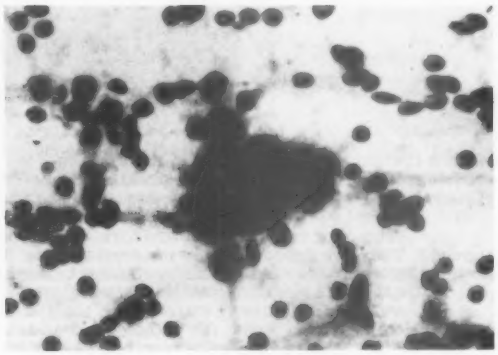

Fig. 4

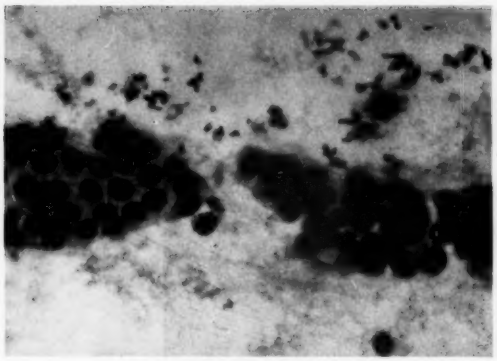

Fig. 5

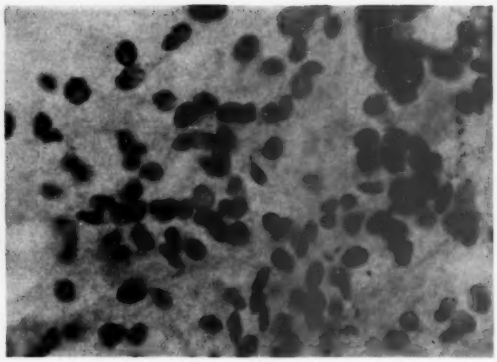

Fig. 6

Fig. 7

Fig. 8

Fig. 9

make a correct diagnosis based on the smears. We found numerous "vermiform bodies" which are described by Ayre and which he thought to be either connective cells from the ulcerated cervical stroma or of parasitic origin.

The elements we noted are formed by elongated and fusiform cells, isolated or grouped in clumps. They are approximately of identical size (5. 5 mμ wide, and 25 mμ long). The cellular outlines are indefinite and the cyanophilic or feebly eosinophilic cytoplasm is pale and difficult to stain. The cytoplasmic structure is granular without any striation. The nucleus is usually absent. When seen, it is central, oval in form, faintly stained with indefinite borders (Fig. 8). All the cells give the impression of necrotic elements. From the pathological slides of these polyps we now know that these cells are exfoliated from a leio-myomalous formation "fibroid" derived from the myometrium just beneath the endometrium and projecting into the uterine cavity. Usually these fibroids do not exfoliate spontaneously.

They are dense and compact tumors with smooth muscle fibers tightly bound together. A ring of endometrium covers the periphery of the fibrosis. Inflammation and chiefly necrosis are necessary to produce the exfoliation of such a tumor. Proteolysis is required to disintegrate and split the smooth fibrous cells (Fig. 9). The only differential diagnosis is the exfoliation of uterine leiomyosarcomas, but the cells are of different sizes, larger and longer than the fibrous cells. All have large abnormal nuclei.

CONCLUSION

"Polyps" represent two very different formations and their cytological expressions are far from similar.

Therefore, it is imperative to have a perfect knowledge of the pathological significance of the term we use, before making a cytological diagnosis.

GUILLERMO TERZANO

Buenos Aires, Argentina

When endometrial polyps are present, endometrial exfoliative cytology is exhibited in the following ways:

A. Normal (Class I) cells
B. Abnormally featured (Class I) endometrial cells due to
   1. irritation because of distortion of the normal endometrium.
   2. erosions, ulcerations, necrosis, etc., in the surface of the polyp or in the surrounding tissues.
C. Clusters of endometrial cells appearing as polypoid formations or as small fragments of the polyp. Anisokaryosis, anisocytosis or normal cytology can be seen.

Erythrocytes are usually numerous.

DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U. S. A.:

The description of vermiform bodies in the presence of submucous fibroids is interesting, but their presence must be rather uncommon since we have never encountered them although we have been searching for them. In my opinion, the cytological diagnosis of endometrial polyps is practically impossible and we have never attempted such a diagnosis. I agree with de Brux that carcinoma never develops in a glandulocystic or fibrous polyp.

COLETTE MARSAN, Paris, France:

For the last two years we have submitted most of the women undergoing gynecological examinations during or after the menopausal period to an intra-uterine smear test.

The smears are obtained by aspiration with a metallic, bent tube, when possible (with women still menstruating) during the days following the menstrual period.

The only contraindications are in cases of infection of the cervix or possible pregnancy.

The cytological responses can be divided into three groups:

1) Clearly negative smears (Class I or II).
2) Clearly positive smears (Class IV or V).
3) Suspicious smears (Class III): showing small clumps of endometrial cells with variable shapes, slightly irregular nuclei and numerous, but usually normal mitoses (Figs. 1 - 2).

   Like Papanicolaou and Pundel, we include in this class, post-menopausal non-suspicious smears which show numbers of endometrial cells, even when they are normal, knowing that they can show the existence of endometrial polyps or hyperplasia, as well as highly differentiated endometrial carcinoma.

Our policy is that the last two categories require, whatever the clinical context, supplementary information: radiological examination or curettage. These procedures have led us to the following conclusions:

1. In most cases, we have not been able to establish final distinctive criteria between endometrial polyps and any other endometrial benign epithelial proliferation, except for the fact that a con-

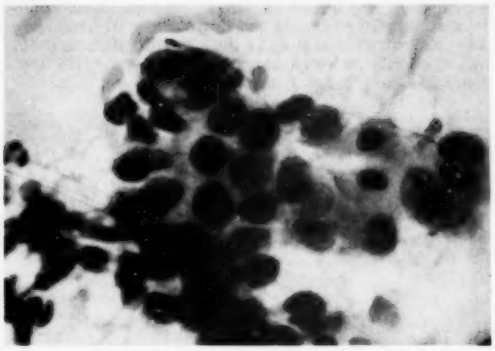

Fig. 1. Small clumps of endometrial cells with regular nuclei but with slight abnormalities in the structure of nuclear chromatin. Two pyknotic, highly suspicious nuclei. It was, in fact, an enormous benign endometrial polyp, occupying the entire uterine cavity.

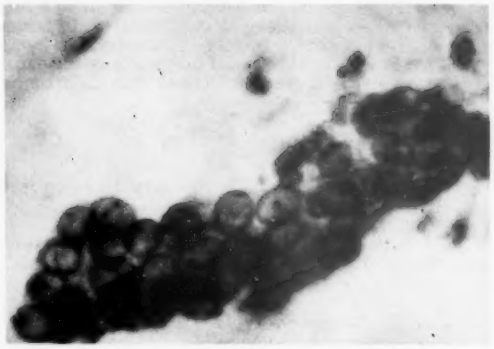

Fig. 2. Clumps of regular endometrial cells, among which only one shows a slightly larger nucleus. This smear had been interpreted as corresponding to an endometrial polyp, but it was in fact a corporal carcinoma.

committant excess of estrogens on the vaginal hormonal smear, would reveal a glandular hyperplasia rather than a single endometrial polyp.

2. If most of the corporal carcinomas are effectively diagnosed by this method, endometrial polyps are, nevertheless, responsible for most of the gynecological false positive smears (Parret, Small and Winn, Bourg, Pundel and Gompel). The complexity of the problems is still increased, in this case by the frequent association of inflammation or metaplasia.

In conclusion: We are convinced that intra-uterine aspirations should, at least in some cases, have no other object than to draw the attention of the clinical technician towards an abnormal epithelial proliferation which might be qualified by the cytologist, by the terms of "probably benign" or "probably malignant" according to the case, and whose exact nature would not be exactly defined by cytology alone. We have not considered the case of submucous fibroids which, in our opinion, is not included among endometrial polyps.

YOSEUP S. SONG, Providence, Rhode Island, U.S.A.:

The cytological features of endometrial polyps are difficult to evaluate. Occasionally, examinations of smears obtained from patients with endometrial polyps often reveal a few groups of endometrial cells with cyanophilic cytoplasm which is slightly vacuolated. The nuclei are small and centrally located with ill-defined nuclear membranes. Nucleoli are generally indistinct. Occasionally the cytoplasm is somewhat elongated. By smear findings alone, it seems extremely difficult to establish a definite diagnostic criteria for endometrial polyps. Frequently, tissue studies on uterine curettings, performed because of suspicious cytology for fundal carcinoma, reveal endometrial polyps without malignancy.

In our series, a small number of cases of endometrial polyps were accidentally found when the tissues were studied. However, corresponding smears quite often did not contain any characteristic cells which would lead us to suspect endometrial polyps.

CLOSING REMARKS

JEAN de BRUX:

In reply to the discussion by Emmerich von Haam: I agree with him on the rareness of vermiform bodies. However, I wish to add that to see them on the smear the submucous fibroid must be necrotic.

In reply to the discussion by Colette Marsan: If endometrial hyperplasia is difficult to distinguish from a differentiated carcinoma, for a trained eye it is relatively easy to distinguish an endometrial polyp from a carcinoma, and one may even diagnose the two lesions when they exist side by side. Based on the clinical signs or on the hysterography, Colette Marsan is perhaps capable of making the diagnosis of fibroids forming a polyp in the uterine cavity. Less versed on this point than she is, I naively thought fibroids entered into the program; I apologize.

GUILLERMO TERZANO:

The comments of von Haam, Song and Colette Marsan complete the subject "Cytology of Endometrial Polyps." I would like to thank them for their fine contributions.

CYTOLOGY OF ENDOMETRIAL ADENOCARCINOMA

HANNES KREMER
Vienna, Austria

Most of the carcinomas of the uterine corpus are of adenomatous nature. According to its structure, carcinoma adenomatosum cylindrocellular, as a rule, sheds large, relatively pale cells exhibiting mostly isokaryosis and only a few pathologic mitoses. According to our experience, cells of the following tumor-like hyperplasias are often difficult to differentiate from these carcinomatous cells: cells of common polyps of the uterine corpus or the cervix cells of polyp-like endometrial proliferations in cases of glandular hyperplasia cells of glandular hyperplasia itself, and finally, cells of an atypical endometrial proliferation in connection with chronic inflammation. Mainly in inflamed processes, highly polymorphic nuclei result from degeneration.

The so-called adenomatoid carcinoma will, because of its structure, shed cells with high-grade polymorphic nuclei, containing numerous pathologic mitoses. Often solid areas, the so-called squamous metaplasias, are worked out by this type of cancer. Cells with centrally located nuclei and the signs of malignancy are therefore sometimes found in smears of this type of cancer. This fact seems to be of importance since squamous cells are more resistant to the degenerative influence and thus are easier recognized in the cytological smear.

The third and very rare type of cancer of the uterine corpus is the solid type. This type and also the squamous cell carcinoma of the uterine corpus did not occur in our material. It might be that cells of such tumorous growths remain better preserved than cells of some adenomatous growth, and, therefore, also more easily diagnosed in the smear. Besides some polymorphy, pathologic mitoses, multiple nucleoli, sharp, bizzare nuclear borders, we, together with other authors, consider the irregularity of the chromatin content of the nuclei of an adenomatous cancer type as the most important criterion of malignancy. The eccentric position of the nucleus, along with well-preserved cytoplasm (the latter often being vacuolated), allows the interpreter of the smear to identify this cell as being of adenomatous origin. Cells from the uterine cavity occur mostly in clusters in the smears. This is true not only for the normal cells but equally so for tumor cells.

The difficulty of detection is demonstrated by the fact that the exfoliated cells of a corporal carcinoma take a relatively long time before arriving in the lower part of the endocervix or the posterior vaginal fornix. They are, therefore, subject to degenerative influences during a certain period of time. Thus changed, they are much more difficult to identify in the smear than cells which were scraped directly from a cervical carcinoma.

In cases with suspicious clinical symptoms for carcinoma of the uterine corpus, cytology is regarded only as an additional method since these cases will undergo curettage of the cervical canal and of the uterine cavity, regardless of the cytologic report.

A negative cytologic report in case of a so-called erythroplakia together with clinical symptoms suspicious for a cervical carcinoma (for example, contact bleeding, reddish watery discharge) allows a therapeutic treatment (electrocoagulation). A negative cytologic result, however, requires other diagnostic procedures when suspicious clinical symptoms for carcinoma of the uterine corpus exist (bleeding in the menopause, irregular uterine bleeding in women over 40 years of age). In these cases, fractionated curettage of the endocervix and uterine cavity is indicated.

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VIOLETTE M. NUOVO, GENEVIEVE DALIAN and ARLETTE SIMATOS
Paris, France

Most of the authors who have investigated the cytological diagnosis of genital cancer have pointed out the difference between the accuracy of the detection of cervical cancer and the relatively high percentage of mistakes in cancers of the fundus.

We have tried to assess the accuracy of exfoliative cytology in the diagnosis of endometrial cancer.

From October, 1949, to January, 1958, 32,333 gynecological patients have been screened. One hundred fifty-five of them had adenocarcinomas of the fundus.

1. In 139 cases the cancer was diagnosed by means of cytology.
   -- 99 had Class IV smears.
   -- 40 had Class III smears.
   -- 12 had false negative smears.
      -- 6 had Class II smears,
      -- 6 had smears which were impossible to type and other smears were requested.

2. In two of these cases the smears were positive and the pathology was suspicious.

3. In 8 cases the smears were positive and the operative specimens negative:
   -- 3 cases of polyps and endometrial hyperplasia.
   -- 4 cases in which no lesions could be found.
   -- 1 cases of endometritis.

In 4 other cases:
   -- 3 cases had only negative endometrial biopsy.
   -- 1 case had a negative hysterography.

Thus the number of false negatives is 12 (8.2 percent) and the number of false positives is 12 (0.03 percent).

It is important to compare these results with the results obtained in the studies of squamous cancer of the cervix.

	No. of cancers in 32,333 cases	False negative No.	%	False positive No.	%
Squamous Carcinoma	544	8	1.4	57	0.17
Adenocarcinoma	151	12	8.2	12	0.03

It is obvious that in cases of cancer of the cervix, the accuracy of cytology is much greater than in detecting cancer of the endometrium. This can be attributed to two reasons: first, lack of exfoliation of endometrial cells; second, the necrosis and degeneration of the cells. The diagnosis is especially difficult in premenopausal women where a normal or hyperplastic endometrium may shed altered cells which can be considered malignant and, conversely, neoplastic. Since there can be a lack of endometrial cell exfoliation, repeated smears give a better accuracy.

	Number of positive or suspicious smears	Percentage of accuracy
First smears:	114	75%
Repeated smears:	139	92%

From the Cytology Department, Service du Pr. Varangot, Maternité de Port-Royal, Faculté de Médecine de Paris, 121 Bd. Port-Royal, Paris, France.

The percentage of errors does not seem the same whether the patient is non-menopausal or menopausal. Among the 151 cases of adenocarcinoma of the fundus:

-- 118 patients were postmenopausal women.
-- 33 patients were normally menstruating women.

Among the false negative smears:

-- 8 were smears of postmenopausal women, representing a percentage of 6.7.
-- 4 were smears of normally menstruating women, representing a percentage of 12.1, which is almost double.

Among the false positive smears:

-- 8 were smears of postmenopausal women, representing as in the false negative smears a percentage of 6.7.
-- 4 were smears of normally menstruating women representing a percentage of 12.1.

In studying the gynecological cytology of glandular carcinoma, we have been struck by two problems:

1. The estrogenic vaginal picture in postmenopausal women with cancer of the endometrium.

We have observed the frequency of a very important pyknosis in the vaginal smears of many postmenopausal women with cancer of the fundus. To appreciate the frequency of this condition, we have compared the respective value of pyknosis in two groups of patients:

Group I – This group includes 89 postmenopausal women, without cancer, who have not been submitted to any hormonal treatment; only 3.5 percent of these patients had vaginal smears showing a Karyopyknotic Index of over 30.

Group II – Only 98 of the postmenopausal women who had a cancer had no hormonal treatment. Thirty-three of them had vaginal smears showing a Karyopyknotic Indices of 30 to 100.

Except for one, these patients were menopausal for:

1 year	2 patients
2 years	4 patients
3 years	3 patients
4 years	1 patient
over 5 years	7 patients
over 10 years	9 patients
over 20 years	3 patients
over 30 years	3 patients

The difference between these two series is very striking. We see that the pyknosis is very important in 33.6 per cent of menopausal women with cancer against 3.5 per cent without cancer.

What is the real significance of this fact? It is still too early to give a satisfactory explanation.

We can assume that this high Karyopyknotic Index is the consequence of the production of a relatively high amount of estrogenic hormone in the postmenopausal women.

However, if this estrogenic hyperstimulation is often observed, it is not a sine qua non condition.

On the basis of these findings, it would be wise that patients whose late menopause suggests an excess of estrogenic production should be submitted each year to a cytological investigation in order to detect an early stage adenocarcinoma.

2. The Class III Problem.

Since the difficulties in the cytological diagnosis of cancer of the fundus are always present in our minds, we may have been more inclined to type the smears as Class III than was necessary. This is also true for any glandular cancer located in the fundus or elsewhere (cervix, tubes). So we have made a comparison between the glandular and the epidermoid Class III.

Glandular Class III.

392 cases were typed as Class III, on first smear.
303 were followed.

-- 171 had negative repeat smears, representing 56 per cent.
-- 55 still had Class III repeat smears, 31 of them had suspicious pathology reports. This represents a percentage of 16%.
-- 77 had positive repeat smears, all confirmed by pathology, representing 25 per cent.

Squamous Class III.

688 cases had Class III smears on first smears.
542 were followed.
-- 222 had negative repeat smears, representing 40 per cent.
-- 171 still had Class III repeat smears of which 97 had suspicious pathology reports. This represents a percentage of 31.
-- 149 had positive repeat smears, all confirmed by biopsy, representing a percentage of 27.

It is obvious that the number of false suspicious smears is greater when the cytologist suspects a glandular cancer than when he suspects a squamous carcinoma. But the different percentages remain in the same range.

The probability of glandular or squamous carcinoma when the first smears are typed Class III is always 25 per cent chance in both cases.

This comparison shows the importance of repeating smears, particularly the Class III smears.

Endometrial carcinoma can be diagnosed with satisfactory accuracy by means of cytology.

The cytological diagnosis of endometrial cancer is more difficult than the recognition of squamous carcinoma of the cervix, especially in premenopausal patients.

As a method of detection of endometrial carcinoma in asymptomatic patients, smears are certainly very valuable, especially in patients with a lengthy duration of hyperestrogenic stimulation.

ERICA WACHTEL
London, England, U.K.

The morphological properties of adenocarcinoma cells are:

1) those of malignant cells in general, with typical malignant nuclear characteristics, i.e., irregular chromatin granules varying in size and shape, nuclear enlargement and anisonucleosis, irregular nuclear outline and hyperchromatism, in that order of importance.
2) the particular features of the endometrial neoplasm manifested by the appearance and structure of the cytoplasm, which is very often foamy in appearance and vacuolized. In contrast to squamous malignant cells ENDOMETRIAL CANCER CELLS ARE USUALLY MUCH SMALLER AND ROUNDER AND SHOW LESS ANISOCYTOSIS.

In addition to the cytological criteria of the single cell the appearance of cell clusters is of particular importance in making a diagnosis. Endometrial adenocarcinoma often exfoliates groups of cells together, a fact which greatly facilitates correct interpretation. In vaginal smears of patients with papillary adenocarcinoma the finding of whole pieces of tumor tissue corresponding to the papillary excrescences of the growth presents no great rarity.

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN Soost, Munich, Germany:

Anisonucleosis and polymorphism of the often greatly enlarged nuclei, as well as their hyper- and polychromatism, must be considered as criteria for malignancy in corporal adenocarcinoma.

In comparison with cervical squamous carcinoma, it is noted that (1) there is an unusually frequent appearance of one or more markedly enlarged nucleoli, (2) there is often marked vacuolization of the cytoplasm, which is rarely seen in squamous carcinoma.

The small, comparatively even shaped nuclei, which are so difficult to differentiate from normal endometrial cells, appear typical of the fully developed adenocarcinoma. The excellent investigations of Violette Nuovo, Genevieve Dalian and Arlette Simatos, as well as the earlier publications of Boschann, Hecht and Ikle, Papanicolaou, Wied, etc., show what outstanding results can be achieved in cyto-diagnosis of corporal carcinoma. We agree with Kremer, however, that cytology alone cannot exclude carcinoma of the uterine body with sufficient certainty. In every case of clinical suspicion of corporal carcinoma, a curettage should be done.

JEAN A. de BRUX, Paris, France:

As most of the main speakers have stated, the cytological diagnosis of endometrial carcinoma is difficult to establish.

The morphological characters of the cells may be split into three categories:

(a) The cells exfoliate in small groups or in clumps, the diagnosis of which is easy. These cells are large and irregular in form. The cytoplasm is more or less cyanophilic and vacuolized. The nuclei are centrally located or pushed toward the border, enlarged in volume, irregular, with prominent nucleoli and nuclear chromatin sometimes rather evenly distributed. Not infrequently there are mitoses.

(b) The cells are most often found singly and have undergone marked degenerative phenomena: in particular, the cytoplasm is very vacuolized, and the nuclei, which are still rather large, are pyknotic and show a lytic tendency. The difficulty of diagnosis is great, for macrophagic and lipophagic cells may assume identical aspects. However, there is a particular cellular element which is often an important diagnostic aid: enormous cells which are macrophaging polynuclear leukocytes. These are often encountered in necrotic and suppurative endometrial cancer.

(c) The difficulty is extremely great when the only elements encountered are inflammatory cells and nuclear debris. In this case one must base one's opinion on indirect signs such as coexisting eosinophilia (related to a hyperplasia of the ovarian cortical stroma, and examinations made after test therapeutic, anti-inflammatory and hormonal treatments.

In spite of these precautions, errors may still be made on atrophic endometrial cells and on cells of the epithelium of an endometrial polyp, modified by chronic inflammation or simply by the existence of a pyometra.

But even granted these difficulties, we find the percentage of Class III presented by Violette Nuovo and co-workers to be excessively high. Even more surprising to us seems the number of cases in which the pathologist reported "suspect" findings on endometrial as well as cervical slides. The pathologist should be the cytologist's conscience and not his pal. This system of classification, in fact, reflects the degree of training, the solidity of the diagnosis and the moral courage of the cytologist and of the pathologist, who should know how to take a firm position.

MANUEL GALBIS, Valencia, Spain:

The only way to secure well-preserved endometrial neoplastic cells is by endometrial aspiration. Even in smears so obtained, the diagnostic difficulties remain and the mistakes in diagnosis are higher than in using cytology in cases of cervical carcinoma.

Often the cellular changes are unremarkable and the cytologic differentiation with cases of endometrial hyperplasia, polyps and inflammatory reactions may be very difficult. Of course, it depends on the histological characteristics and degree of differentiation of the adenocarcinoma, as Kremer reports. We have had 5 cases of false-positive smears in 37 cases of hyperplasia and/or polyps, and 4 false-negatives out of 18 cases of adenocarcinoma. Thus, our percentage of errors is high and we agree with Kremer in his statement that a negative smear in a woman with suspicious symptoms requires further diagnostic procedures.

Violette Nuovo reports a relatively low percentage of errors, using, apparently, the vaginal smear. We have not obtained this accuracy and we do not share her conclusions.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.

We are in hearty agreement with Violette Nuovo and co-workers that the cytological diagnosis of endometrial cancer is more difficult. One of the reasons for this difficulty was correctly pointed out by Kremer. In our laboratory much emphasis is placed upon the appearance of malignant cells in cell clusters, as stressed by Erica Wachtel, and the very common finding of high estrogenic smears in menopausal women having endometrial carcinoma. In fact, we regard this phenomenon as a rather typical pattern, and it is very uncommon to have carcinoma of the fundus in an old patient with an atrophic vaginal smear. In this respect our figures are even more striking than those quoted by Violette Nuovo.

JULIETA C. de LAGUNA, Mexico, D.F., Mexico:

We agree with Kremer in the necessity of performing curettage of the uterine cavity in clinically suspected cases. On the other hand, we would like to emphasize that in any case with a positive smear for carcinoma of the uterine body, cytology is of paramount importance. In our group of 72 carcinomas of the endometrium, which are discussed at length elsewhere in this Symposium, 8 of them were detected by cytology.

JOSÉ MARIA MEZZADRA and GUILLERMO TERZANO, Buenos Aires, Argentina:

There is a general agreement that it is more difficult to describe the exfoliative cytology of endometrial carcinoma than the exfoliative cytology of the squamous cell carcinoma of the cervix. We must also admit that it is not frequent that the cytological diagnosis of a lesion of the endometrium has been correctly made. Apart, of course, from the cases in which a positive or a negative diagnosis is obvious, there are always those cases in which the cytologist would feel much happier if he were

allowed to report the case as Class III suspicious, because he honestly dares not go beyond Class III, and neither can he report the case as negative.

Early carcinomas desquamate very few cells, and the mature differentiated carcinomas shed off cells that do not show convincing evidence of malignancy. A 32 year old woman (Mrs. B., Case No. 7092), because of abnormal bleeding, had an endometrial biopsy that was reported as suspicious. Three months later she came back to the hospital in very good health with no abnormal bleeding at all. In the smears (endometrial aspirations), though they were rich in endometrial clusters, no cell nor any cell cluster was found showing conclusive evidence of malignancy, and the smears were classified as Class III. Because of the previous pathological report and fearing a false negative, a curettage was indicated and an adenocarcinoma was diagnosed.

Another false negative report we would like to mention is the case of a 60 year old woman (Mrs. C., Case No. 694) who complained of bleeding 8 years after her menopause. A large number of histiocytes and clusters of Class I and Class II endometrial cells, made us think of an inflammatory process rather than a neoplasm. The smears were reported as Class III, suspicious. Pathological report: adenocarcinoma of the endometrium. When the smears were reviewed, we had to admit that the carcinomatous cells were mistaken for histiocytes.

On the contrary, it must be remembered that degenerated cells, ill-preserved cells, necrotic cells, cells with large vacuoles and signs of phagocytic activity may be present in smears which present quite a confusing cytologic picture. Also the presence of abnormal endometrial cells in smears obtained during the process of regeneration of the endometrium, such as happens after a curettage, an abortion, a delivery, etc., is similarly confusing.

GEORGE H. ROMBERG, White Plains, New York, U.S.A.:

It is assumed that the cytological criteria presented in all three papers refer to vaginal and cervical smear findings since no mention is made of the endometrial aspiration technique. I would like to ask these essayists to comment on their experiences with this technique and to state whether they feel it increases the accuracy of the cytological diagnosis of endometrial adenocarcinoma. Does Kremer believe that endometrial cells obtained by the aspiration technique show a better state of preservation than those found in vaginal smears? I would like to ask Erica Wachtel to comment on the finding of leukocytic infiltration in clusters of glandular cells in cytologic smears. Does she consider this finding helpful in making a correct diagnosis? Our own observations correspond with those made in Violette Nuovo's paper, that persistent hyperestrinism occurs frequently in post-menopausal females with proven endometrial adenocarcinoma.

LUBA A. TURNBULL, Montreal, Canada:

The reason for a low diagnostic accuracy in adenocarcinoma of the endometrium is the difficulty of the diagnosis. Endometrial cancer cells show a large variation of cytoplasm and nuclei. They are quite small in diameter and appear in large or small clusters in vaginal secretion, but could be absent in vaginal discharge in cases of endocervical stenosis. Endometrial cells seen in vaginal smears are some times badly degenerated or necrotic, and then we are not able to tell whether they are positive or negative.

As Violette Nuovo mentioned, there is, however, higher accuracy in postmenopausal women than in menstruating patients. I believe that this is due to the fact that smears of postmenopausal patients are especially studied for adenocarcinoma. We are accustomed to thinking of endometrial adenocarcinoma as an old-age disease and, therefore, do not suspect endometrial cancer in young, menstruating women.

In the record book where we daily enter all cancer cases diagnosed in our laboratory, we see 31-32 year old patients with adenocarcinoma of the endometrium. This means that every smear with abnormal bleeding, spotting or leukorrhea should be studied very carefully, and it is better for the cytologist to recommend a curettage in undetermined cases with symptoms than to let the patient go home trusting in a negative test, and then to return in 6 months with the clinical symptoms of endometrial carcinoma.

CLOSING REMARKS

HANNES KREMER:

In reply to the discussion of Romberg:

I do believe that endometrial cells obtained by the aspiration technique are better preserved than those in the vaginal smear.

In reply to the discussion of Julieta de Laguna:

There is no doubt of the importance of cytology also for the detection of carcinoma of the body of the uterus. The accuracy, however, in detecting adenocarcinoma of the uterine corpus is not as high as in carcinoma of the cervix.

In reply to the discussion of Bickenbach and Soost:

As was expected, other authors also emphasize the importance of curettage of the corpus of the uterus in cases suspicious for corporal carcinoma.

VIOLETTE M. NUOVO:

I agree with Romberg's assertion. I should have mentioned that there were 63 endometrial aspirations among the 139 cases in which the cytology was positive for adenocarcinoma. Further details are given in the discussion of "Endometrial Aspiration Technique" and "Vaginal Smears for the Detection of Endometrial Carcinoma."

I would answer Galbis' remarks similarly.

To de Brux, I would like to add that it seems inconceivable to give a moral value to the Class III category. Class III for us is based only on cytological criteria to which we are implicitly faithful. We do not blend them with ideas of courage and moral dignity. As for the numerous pathologists who see our cases, although we do not know all of them personally, we assume that they have the same attitude.

We do agree with the comments of Mezzadra and Terzano, of Bickenbach and Soost and of von Haam, as well as with those of Turnbull.

ERICA WACHTEL:

I have no comments to add except perhaps the observation that most discussants concentrated on arguing about diagnostic accuracy (which is dealt with in a separate chapter in this volume) rather than dealing with cytologic criteria of endometrial adenocarcinoma.

With regard to Romberg's question on the significance of leukocytic infiltration in clusters of glandular cells I would like to point out that this finding is not pathognomonic for endometrial cancer, but also occurs in other cases such as ulcerating endometrial polyps, sloughing of the endometrium, endometritis, etc.

Finally, I would like to ask Professor de Brux, who is not only a leading cytologist but also an eminent pathologist, how he manages to follow his own doctrine of "the pathologist being the conscience of the cytologist and not his pal." If he succeeds in accepting this "obiter dictu," I imagine his life must be pretty difficult.

CYTOLOGY OF ENDOMETRIAL ADENOACANTHOMA

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

Adenoacanthoma of the uterus represents one of the less common histological types of carcinoma of the endometrium in which islands of "squamous metaplasia" appear among the atypical glandular epithelium of endometrial adenocarcinoma. According to Novak (1), this change is more often seen among the more differentiated adenocarcinomas of a lesser degree of malignancy, which explains the better prognostic outlook of this type of tumor.

In order to make a cytological diagnosis of adenoacanthoma, malignant cells of the squamous type as well as of the glandular type must be recognized in the vaginal smear, although Graham (2) emphasizes that sometimes only one type of cell will exfoliate, while the biopsy will show the typical picture of this "mixed type" of carcinoma. The other possibility, namely that both types of malignant cells are present in the vaginal smear, while the biopsy may only show one type of carcinoma, can be explained by the irregular distribution of the squamous cell nests among the adenocarcinoma and the size and location of the biopsy. In the ten years of our cytological experience we have encountered this type of tumor nine times and were able to make the correct cytological diagnosis in six cases. During this period we have encountered sixty-one cases of adenocarcinoma of the endometrium, which would suggest that this type of tumor is more frequent than is commonly anticipated. It appeared approximately once in every 14,700 patients examined cytologically. The age group of the patients varied between 53 and 72, which is considerably higher than the series of sixteen cases published just recently by Novak and Nalley (3). The parity varied between 0 to 4 with only two abortions in the entire group. Seven of the nine cases had reached the age of menopause anywhere from six to twenty years before discovery of the lesion. Two still menstruated at the ages of 53 and 55 but the periods were described as weak and irregular. In only three of the nine cases was the presence of a malignant tumor suspected clinically. All three spotted irregularly from two weeks to three months. The other six cases went to their physicians for a routine checkup and three of them were patients of the large Cancer Survey Project conducted presently among women of Franklin County through the joint efforts of the Ohio State University and the National Cancer Institute. Two of the patients had only vaginal smears, three of the patients had vaginal smears and cervical scrapings, and four of the patients had vaginal smears, cervical scrapings and endocervical aspirations.

Interpretation of Cytological Findings:

Seven of the patients showed a hormonal response corresponding to age and length of menopause. In two patients a marked involutional atrophy was present, while two patients, ages 55 and 60, showed an increased estrogen effect corresponding to that of young and menstruating women. Leukocytes were present in markedly increased amounts in three cases and absent in three cases. Red blood cells were present in moderate quantities in five cases and absent in four. A cytological diagnosis of malignancy was made in six cases. In all six cases, the true character of the tumor was recognized by the presence of malignant squamous cells as well as malignant glandular epithelial cells. The malignant squamous cells appeared in small groups and in two cases malignant pearly bodies were present. The squamous cells were of the more differentiated type and showed bizarre-shaped cells with highly keratinized cytoplasm and large pyknotic nuclei (Fig. 1). They were less numerous than the malignant glandular cells but on the other hand were much more easily detected. The malignant cells were of the large glandular cell type with mucous vacuoles and large marginal nuclei. In some of the cases one or two of the malignant squamous cells were surrounded by clusters of malignant glandular cells (Fig. 2). The correct cytological diagnosis was made in six cases and missed in three cases, which gives a none too favorable accuracy of 66 per cent. The vaginal smear proved to be the most accurate method of collecting material, or was at least as accurate as the endocervical aspiration. The cervical scraping proved less accurate. In two cases no malignant cells were found in the cervical scrapings, while they were present in the vaginal smears and endocervical aspirations. This experience is similar to our experience with glandular carcinoma of

the endometrium in which we found the aspiration from the vaginal pool a much more accurate method of collecting material. In three of our cases we failed to make a cytological diagnosis, despite the fact that in two cases suspicious bleeding was present. In one case, that of a 69-year-old white woman, the tumor was discovered after hysterectomy for uterine prolapse. Dissection of the specimen revealed a stenosis of the internal os which prevented the exfoliated cells from draining through the cervix. In all nine cases simple hysterectomy was performed and only one patient has died thus far from extension of the tumor into the pelvic organs and urinary pathway obstruction. In the histological evaluation a diagnosis of adenoacanthoma was made because of the presence of numerous islands of metaplastic and highly atypical cell epithelium. In most of the cases the invasive portions of the tumors were predominantly glandular in character, while the squamous cell islands were found more toward the endometrial surface of the tumor surface. In one case who is still living, both types of epithelium metastasized to the deep iliac lymph nodes. We are in agreement with the experience of Novak and Nalley that adenoacanthoma represents a less malignant and slower growing neoplasm in the uterus with a better over-all prognosis. Three of the cases underwent irradiation treatment before hysterectomy and the cytological picture changed in all three cases with marked increase of bizarre squamous cells anywhere from two to three weeks after irradiation. Similar changes were recognized in the islands of squamous cells of the removed specimen. It seems that irradiation had a marked keratinizing effect with increased metaplasia of the malignant cells toward the squamous cell type.

In summary, we feel that the cytological diagnosis of adenoacanthoma of the uterus can and should be made whenever malignant squamous cells and malignant glandular cells are recognized in the vaginal smear. The only other lesion which would produce a similar picture is the simultaneous occurrence of squamous cell carcinoma of the cervix and an adenocarcinoma of the endometrium which is very rare indeed and which we encountered only once in our entire experience.

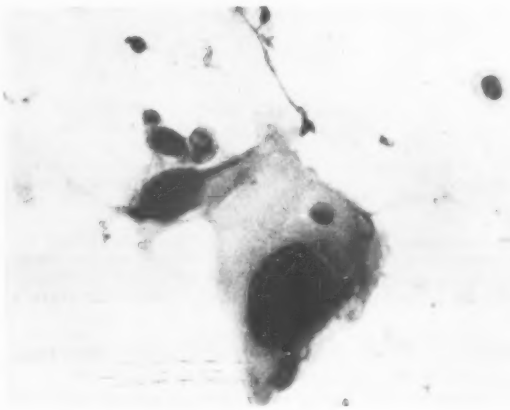

Fig. 1. Vaginal smear showing two malignant and bizarre squamous epithelial cells and one malignant glandular cell. (x 440) (E. von Haam)

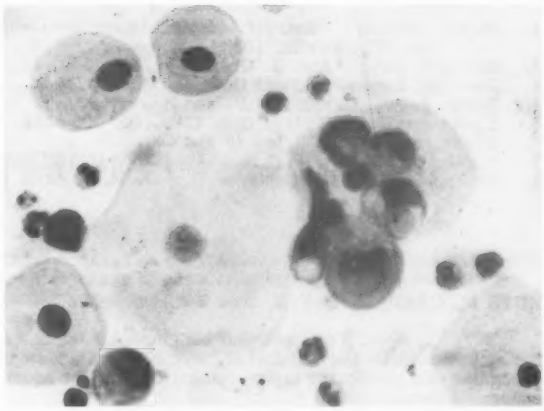

Fig. 2. Vaginal smear showing cluster of malignant glandular cells and one malignant squamous cell. (x 440) (E. von Haam).

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LEOPOLD G. KOSS
New York, New York, U.S.A.

An adenoacanthoma, by definition, is a malignant tumor, primarily of glandular origin and structure, but with a substantial squamous or epidermoid component. Such tumors occur mainly in mucous membranes or glands which are located anatomically in areas where the glandular and squamous epithelium either meet or are not too far apart. The endometrium and the cardia of the stomach belong to areas where adenoacanthomas occur with fair frequency. Other sites of occurrence include the rectum, gall bladder, etc. It is, of course, logically permissible to consider all of the so-called muco-epidermoid cancers, including the malignant tumors of the salivary glands and the uterine cervix, as variants of adenoacanthoma. The occurrence of this tumor type indicates, to my mind, multiple developmental potentialities of the epithelium.

Adenoacanthoma of the endometrium, or at least the presence of well-differentiated squamous components in endometrial cancer, is, in the author's experience, not too uncommon and may be readily found in at least one out of every ten endometrial cancers. Robert Meyer (1) speaks of the relative frequency with which squamous epithelial components may be found in endometrial cancer. Marked unilateral development of the squamous component may produce benign lesions of the endometrium (2) or pure forms of squamous carcinoma of the endometrium of which there are a few examples in the literature (3). Elements of benign squamous epithelium may be found within the endometrium in the absence of tumor (4). Meyer (5) mentions the presence of squamous epithelium within the endometrium of young children and in benign hyperplasias.

In smears the correct diagnosis of an adenoacanthoma is a spectacular and yet a relatively simple task. These tumors shed two types of cells, some of which have all the characteristics of an endometrial adenocarcinoma and some, those of squamous carcinoma. Contrary to the widespread impression that the squamous component of an adenoacanthoma is a benign one (6), it has been the author's experience, that the squamous component is that of carcinoma, capable of metastases. This view is also shared by others (7).

The differential diagnosis of endometrial adenoacanthoma comprises primarily endocervical cancers, which may also have both the glandular and squamous components. In fact, malignant, mucous-producing glandular cells may be found with fair frequency in essentially epidermoid cancers of endocervical origin. This fact has been observed by cytologists for a long time and has usually been considered a form of degeneration of epidermoid cells.

Within the cervix, the two tumor components may be either mixed or topographically separated. In this situation, the correct interpretation of smears as to the primary tumor site may prove to be impossible.

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DISCUSSION

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

I am pleased to see that both authors emphasize the fact that the squamous component of this tumor is malignant and not merely atypical squamous epithelium. It would, of course, be impossible to recognize this particular lesion cytologically if the squamous component were not typical of differentiated squamous carcinoma.

F. A. IKLÉ, St. Gallen, Switzerland:

In our experience adenoacanthomas are rare. Among 46 adenocarcinomas of the endometrium during the last two years, we had only one such case. This was also the only case among 4025 smears

taken during the same period of time. The interpretation of the cytological findings was adenocarcinoma of the endometrium. Vaginal smears and intra-uterine aspirations proved to be more accurate than cervical scrapings in this particular case.

The biopsy was interpreted as highly undifferentiated squamous cell carcinoma, probably of the cervix. Because of the discrepancy between the histological and the cytological findings, an adenoacanthoma of the endometrium seemed probable.

The hysterectomy was done only after preoperative irradiation with intra-uterine radium as per routine (14 elements of 10 mg. for 12 hours: 1650 mg. hours). The interpretation of the smears taken 1, 2 and 3 weeks after irradiation proved to be difficult and inconclusive.

The removed uterus showed an invasive adenoacanthoma of the endometrium which was almost entirely necrotic and highly "sterilized" by radium.

The adenoacanthomas belong to the more differentiated adenocarcinomas of the endometrium. This type of tumor has neither prognostic nor therapeutic peculiarities. The cytological diagnosis is only of academic interest.

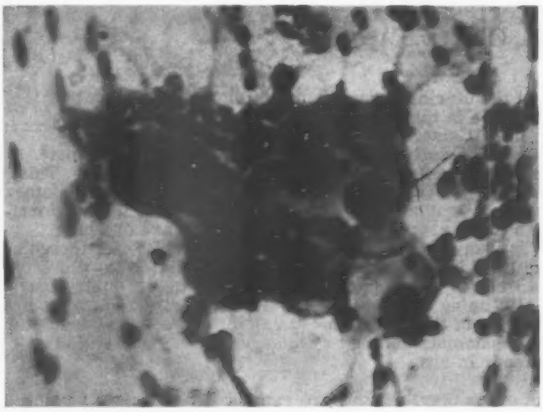

Fig. 1. Intra-uterine aspiration smear from an adenoacanthoma of the body of the uterus. Note: Cluster of atypical cells cytologically interpreted as endometrial adenocarcinoma. Characteristics of squamous carcinoma are absent. (F. A. Iklé)

JULIETA C. de LAGUNA, Mexico, D.F., Mexico:

Out of 91 cases of histologically proven carcinomas of the endometrium, 10 have been of the adenoacanthoma variety. We agree with the authors that their exfoliated cells represent both the adenocarcinoma and squamous carcinoma types. However, in our cases there has been a clear cut predominance of one of these types of cells, most of the diagnoses having been erroneous concerning the histological type. Thus, our findings have been as follows:

Positive V, Adenoacanthoma	3 cases
Positive V, Adenocarcinoma	3 cases
Positive V, Squamous carcinoma	2 cases
Positive IV, Carcinoma	1 case
Doubtful III	1 case

One of the cases diagnosed as squamous carcinoma had a complete invasion of the parametria.

OLAF T. MESSELT, Oslo, Norway:

I can only say that I am in agreement with what the two main speakers have said about this subject. I agree with Koss that the squamous component in cases of adenoacanthoma is that of carcinoma and not merely metaplasia or a benign alteration of the squamous cells.

I also have had the same experience as von Haam that the malignant squamous cells in these cases usually are of a more differentiated type showing keratinization and sometimes "pearl" formation.

CLOSING REMARKS

EMMERICH von HAAM:

My thanks to Drs. Julieta Laguna, Ruth Graham, Messelt and Iklé for their remarks. I do agree that cytological diagnosis of adenoacanthoma is of purely academic interest.

CYTOLOGY OF THE IRRADIATED UTERINE CAVITY

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

Little is known concerning the cytology of the uterine endometrium after radiation. Ruth Graham (1) was unable to observe any definite difference in the endometrial cells which could be attributed to radiation except for a slight increase in cell size. Smolka (2) mentions Mohr's "Intermediaerreaktion" and "Finalreaktion" as one method of classifying the cellular changes but feels that it is impossible to use those changes for any purposes of cytological standardization or prognosis. This may not be surprising since equal uncertainty exists in the evaluation of the histopathology of the endometrium following radiation (Stowe) (3). In the "adequately" radiated uterus, the endometrium is transformed completely into a layer of hyaline necrosis with a loose granulation tissue on the surface of which occasionally swollen epithelial cells can be seen. In less severely irradiated uteri, a number of irregular gland spaces can be noted in this granulation tissue lined by distorted vacuolized epithelial cells with enlarged and pyknotic nuclei. With even smaller doses, the endometrial stroma with its glandular structures remains intact but the individual epithelial cells lining the surface as well as the glands show changes which first consist of simple swelling with vacuolization of the plasma but which later lead to a considerable degree of cellular dysplasia. In this stage, the endometrium may even assume the picture of atypical focal endometrial hyperplasia identified with cancer in situ.

The material used for our study consists of six uteri which were removed one to eight months following radiation therapy. The patients ranged between 45 and 67 years of age and three were still menstruating while three already had reached the menopause. In two cases, the diagnosis of carcinoma of the cervix was made while three patients had histologically proven carcinoma of the endometrium and one patient was treated for suppression of ovarian function in carcinoma of the breast. Two patients received only x-ray treatment to the mid-pelvis, one patient received intrauterine radiation with radium and three cases received a combined treatment of local application with radium and x-ray therapy to the pelvis. Dosages varied between 3,000 and 6,000 mg. hours of radium and 2,000 and 4,000 roentgen units. Smears were obtained by intra-uterine and endocervical aspiration during and after therapy. The removed uteri were opened and smears were prepared by scraping gently the exposed endometrial surface. All smears were stained with the Papanicolaou method.

Acute Radiation Changes

Acute radiation changes could be recognized in the endometrial cavity as early as two weeks after radiation therapy had started, but were in many cases delayed from six to eight weeks. The earliest changes consisted of vacuolic swelling of the endometrial cells which produced a well-distinguishable clear zone of cytoplasm around normal appearing nuclei (Fig. 1). The cells stained pale blue with the Papanicolaou stain and, because of the increase of size, resembled endocervical cells. If tumor cells were present, they also participated in the change and sometimes assumed the shape of signet ring cells (Fig. 2). Exfoliation of irradiated and endometrial cells as well as of irradiated tumor cells seemed greatly enhanced and the endometrium appeared invaded by a varying number of acute inflammatory cells. In uteri with heavy radiation, the epithelial cells, including the tumor cells, disappeared from the endocervical aspirate within three months and only histiocytes, leukocytes and red blood cells remained. Cervical stenosis and scarring made an intra-uterine aspiration practically impossible after the third month following completion of radiation therapy and even endocervical aspirations proved quite difficult.

Chronic Radiation Changes

In the less heavily irradiated uteri, the vacuolic changes of the endometrial cells slowly disappeared and those cells were replaced by cells which appeared larger, somewhat bizarre and possessed enlarged, pyknotic nuclei (Fig. 3). At this stage, there appeared giant cells, which were interpreted by us as Smolka's pseudo-giant cells created by the confluence of altered endometrial cells (Fig. 4). These cells obviously were derived from the dysplastic endometrial glands alluded to in the previous paragraph.

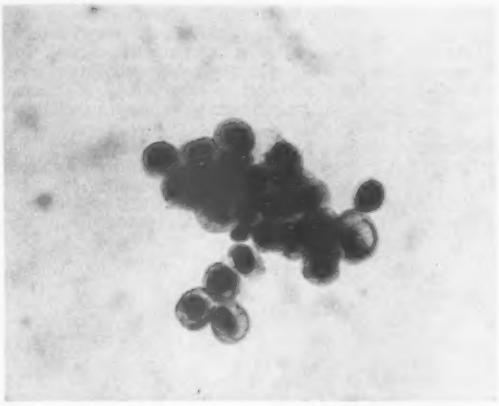

Fig. 1. Endometrial cells showing acute radiation changes. Intra-uterine aspiration 2 weeks after onset of radium therapy. (x440)

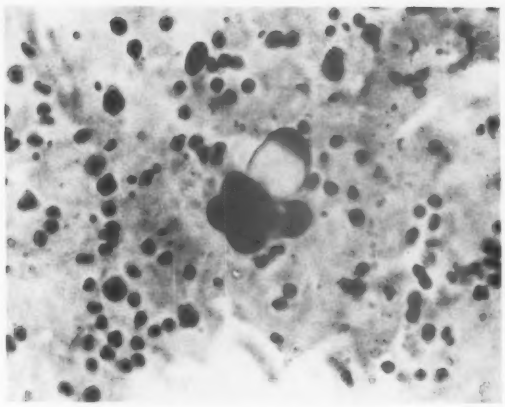

Fig. 2. Malignant endometrial cells showing acute radiation changes. Endocervical aspiration one month after onset of cobalt^{60} therapy. (x440)

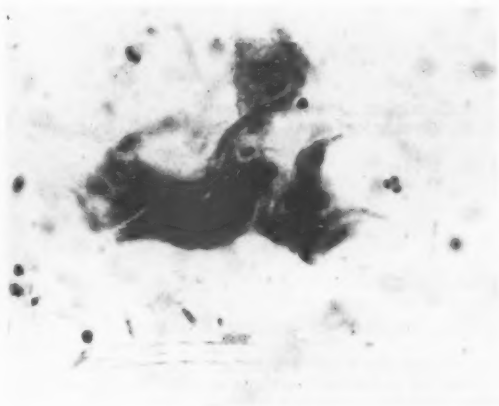

Fig. 3. Atypical endometrial cells from a uterus irradiated for carcinoma of the cervix with 4,500 mg. hours cobalt^{60} and 3,000 r. Endocervical aspirate 3 months after termination of therapy. (x440)

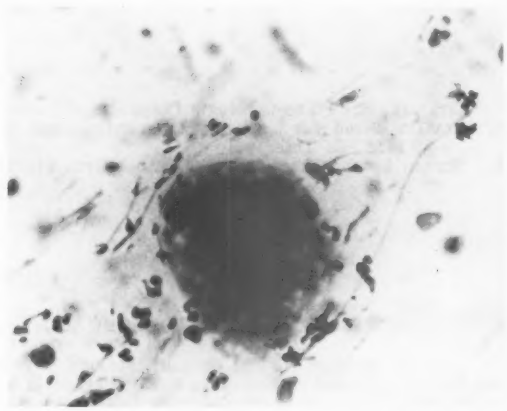

Fig. 4. Pseudo-giant cell from uterus irradiated for adenocarcinoma of the endometrium with 4,000 r. Surface scraping 5 weeks after termination of therapy. (x440)

Surface scrapings from the removed uteri produced also the atypical endometrial cells depicted in Figures 5 and 6. The cells appeared either pale and elongated or assumed bizarre ameboid shapes. Leukocytes and histiocytes were constantly present in large numbers while red blood cells were scarce. Tumor cells were always absent from the irradiated endometrial surface in spite of the fact that they could be discovered sometimes in the deep lymphatic spaces of the uterine wall. For this reason, we considered it highly unreliable to use the disappearance of malignant endometrial cells as a prognostic criterion for the therapy.

We tried also to investigate if any of the cell changes discovered could have any prognostic meaning and have been disappointed in this respect. The number of endometrial cells recovered is always small and the cytological recognition of cells is often difficult. For this reason, a quantitative evaluation of radiation changes proved unreliable. We feel, however, that efforts to relate certain cytological changes with the dosage levels of radiation should be continued; although at the present time, the author feels unable to make any valid correlations. No differences were noted in the endometrial cytology of the irradiated uterus with regard to the type of radiation used.

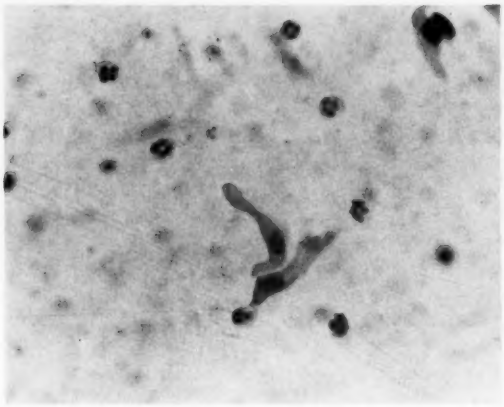

Fig. 5. Atypical endometrial cells from uterus irradiated for carcinoma of the endometrium with 6,000 mg. hours radium and 4,000 r. Surface scraping 4 months after termination of therapy. (x440)

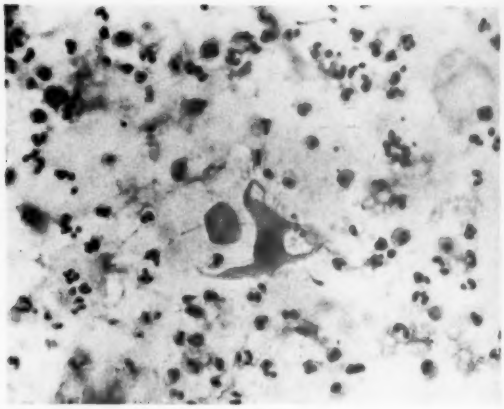

Fig. 6. Atypical endometrial cell from uterus irradiated for carcinoma of the cervix with 3,000 mg. hours cobalt^{60} and 4,000 r. Surface scraping 8 months after termination of therapy. (x440)

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DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

We studied the endometrial cytology in a series of patients with cervical carcinoma before and during combined radium-deep x-ray therapy (3 intra-uterine radium insertions of 2000 mg Eh each at intervals of 10 - 14 days, concurrent deep x-ray therapy with 300 r OD in 4 fields).

We saw the same change in the endometrium as those described by von Haam: enlargement of the cells and the cell nuclei, often pyknosis of the nuclei with indefinite structural features and frequent vacuolization of the cytoplasm. In many cases the cell changes due to irradiation could already be seen 10 - 14 days after the first radium insertion. In other cases these changes could only be seen after the second or third insertion.

Histologically the recognition of these endometrial changes due to irradiation is much easier and more obvious than by the cytological smear, as could be shown in a few comparative histological examinations of scrapings obtained by curettage.

CONSTANTIN HEROVICI, Paris-Villejuif, France:

It is obvious that the cytoprognosis of irradiated endometrial carcinoma is far more difficult than that of carcinoma of the cervix. There is difficulty in taking samples, as one has to pass through an isthmus which may be obstructed under the effects of radiation and which, even under normal conditions, enables one only to take blind specimens consisting mainly of red blood cells, histiocytes, some necrotic cells and a very few patches of the endometrium that remain in good condition. Furthermore, because of their own particular characteristics, glandular epitheliomas of the uterine body are less radiosensitive than those of the cervix, without, however, reaching the state of radioresistance which was attributed to them in the past. This was derived from the fact that an irradiated endometrial cell under the effects of radiation does not show the same spectacular morphological changes as would appear in vaginal or cervical epidermoid types.

Therefore, I agree with von Haam concerning the difficulties in interpreting the reactions of endometrial cells to irradiation. On realizing the deficiences encountered with morphological cytoprognosis, we have used since 1954, cytochemical techniques to study the metabolism of irradiated endometrial cells.

The study of a series of 16 cases of endometrial carcinoma, treated identically, first by radium (according to the method practiced in Stockholm), and then, second, by surgery (hysterectomy with lymphadenectomy), enabled me to compare the actual evolution two years after treatment had been completed with the prognosis that had been made at the end of the treatment from our cytochemical examinations. The most important change observed was the decrease of desoxyribonucleic acid together with the increase of ribonucleic acid in the cytoplasm.

It seems possible to me, and much easier, to make a favorable prognosis when all the cells in the specimen show this post-therapeutic cytochemical anomaly and an unfavorable prognosis when only one patch of cells shows nuclei that are rich in DNA, than to hesitate on the subtilities of an irradiation reaction which is not very obvious in the region of the glandular cells, and, from these, establish interpretation percentages which are more or less subjective.

CLOSING REMARKS

EMMERICH von HAAM:

The cytochemical evaluation of endometrial radiation changes as advocated by Herovici opens a new approach to this difficult problem. In spite of its inherent tediousness and technical difficulty, quantitative cytochemistry must be recommended if it yields better diagnostic results than the admittedly inadequate morphological examination.

CYTOLOGY OF CARCINOMA IN SITU OF THE ENDOMETRIUM

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

Hertig (1) based his concept of carcinoma in situ of the endometrium upon the observations that it later develops into invasive carcinoma, that it precedes the invasive stage of malignant endometrial polyps and that it persists until succeeded by carcinoma. Speert (2) described a pre-malignant phase of endometrial carcinoma which he called atypical or adenomatous hyperplasia and his histological description for this lesion is practically identical with Hertig's carcinoma in situ. TeLinde (3) emphasized that many lesions diagnosed as atypical hyperplasia or adenomatous hyperplasia are really endometrial carcinoma and cited numerous instances where endometrial carcinoma was preceded by atypical hyperplasia for several years. Edmund Novak (4) considered endometrial carcinoma an exaggerated and extreme stage of post-menopausal hyperplasia and described 36 cases of this condition which showed identical histological features in the curettage biopsy as seen in adenocarcinoma. However, in the last edition of his book, the late Emil Novak (5) still thought the use of the term carcinoma in situ "not very useful" and considered it much wiser to speak of atypical hyperplasia in regard to this type of lesion.

This paper is not the place to go further into the arguments of the correct nomenclature of the lesion, important as it may be for statistical and therapeutical considerations. The author is convinced after studying the extensive literature on this subject that all names suggested pertain to the same identical lesion, the cytology of which is the subject of this discussion. Material for this study consisted of 16 cases in which hysterectomy was performed after previous cytological investigation followed by endometrial biopsy. The patients ranged in age from 52 to 68. All had been in menopause for three years or longer. In 7 patients occasional spotting had been observed up to six months before the cytological examination. In all 16 cases suspicious cells were present in the cervical scrapings. In 10 cases where endocervical aspirations were performed and in 4 cases where intra-uterine aspirations were performed, similar cells were found. Hysterectomy was recommended in all cases following an endometrial biopsy which showed lesions identical with those referred to as "atypical hyperplasia" or "carcinoma in situ" of the endometrium. Gross examination of the removed uteri showed no evidence of neoplasm. The endometrial surface appeared either smooth, rough or hemorrhagic. The histological study of numerous sections failed to reveal invasion of the myometrium by the atypical endometrial glands, although no serial sections were examined. We agree with Novak that this type of examination does not rule out invasiveness, but neither does the histological method by which we usually diagnose carcinoma in situ of the cervix.

The cells found were interpreted as atypical and probably malignant endometrial cells. They were of irregular size, showed abnormal shapes and were usually larger than the normal non-secretory endometrial cells (Fig. 1). They were considered atypical whenever the nuclear-cytoplasmic ratio appeared unchanged (Fig. 2), but were considered malignant whenever the nuclear-cytoplasmic ratio was increased (Fig. 3). Some endometrial cells appeared quite round and stained eosinophilic, others could not be differentiated from cells found in invasive carcinoma (Fig. 4). Differences between the cytological findings in this group from those present in invasive carcinoma were more quantitative than qualitative in nature. Red blood cells and leukocytes were usually absent or present only in small numbers in vaginal pool aspirates. The atypical cells appeared only singly or in small groups and there was little evidence of cellular necrosis or cellular cannibalism. Giant cells were absent. Naked malignant nuclei, which we observe so often in advanced endometrial carcinoma, were inconspicuous. Malignant squamous cells as observed in adenoacanthoma were absent.

In summary, we feel that carcinoma in situ of the endometrium gives a cytological picture similar to invasive adenocarcinoma, with the exception of certain quantitative differences which we do not consider sufficient to warrant a cytological differential diagnosis between both lesions.

The question as to what constitutes endometrial carcinoma in-situ, as differentiated from abnormal or atypical or adenomatous hyperplasia (5) is not an easy one to answer. No matter how much has been written on this subject, this definition is primarily a matter of experience that can be, however inadequately, described but which cannot be taught. Carcinoma is a pattern of cells and tissues which has been recognized as capable of invasion and metastases. It is obvious that this experience varies from individual to individual and from institution to institution. No matter how astute the observer and how vast his experience, a certain number of patterns shall remain in doubt – as witnessed by the much quoted statement of Robert Meyer apropos an endometrial lesion: "kein Karzinom, aber besser heraus."

My personal experience agrees with that of TeLinde and coworkers (6), and I believe that most of the endometrial lesions of dubious nature represent forms of early carcinoma.

The writer had the opportunity to observe cytologically and in tissue sections one dozen lesions that were diagnosed either as in-situ or very early invasive cancer of the endometrium. This limited experience does not allow any generalized statement pertaining to the cytologic findings. All of the patients falling into this category have been clinically post-menopausal, yet the make-up of the smears nearly invariably indicated an abnormally high degree of maturation of the squamous cells and presumably an abnormally high estrogenic activity. Contrary to advanced invasive cancer of the endometrium (7), the background of the smears was generally free from blood, either fresh or fibrinated, while some histiocytes were usually present. The cancer cells were as a rule very few, very inconspicuous and difficult to find. The individual cancer cells were usually not significantly larger than normal endometrial cells nor was their grouping of any particular diagnostic assistance. As is the rule in cancers in general, single cells were always available for inspection. The only truly significant cytologic finding in this group of patients, was the presence of moderate degrees of nuclear abnormality, especially multinucleation and the presence of abnormally large and occasionally multiple nucleoli. When the grouping allowed a comparison of individual cells, considerable degrees of anisonucleosis could be observed. Nuclear hyperchromasia was not observed in the author's experience. The feature of infiltration of the cytoplasm of endometrial cancer cells by leukocytes, frequently so prominent in invasive endometrial cancer (7), was occasionally observed. In a case of adenoacanthoma in-situ, abnormal squamous cells were observed as well. In summary, the cytologic findings were inconspicuous and difficult to recognize.

It is likely that the relative success which this laboratory has been enjoying within the last few years in detection of very early carcinoma of the endometrium is in a way due to a very poor performance in the years before. When, in the year 1954, it became obvious that the laboratory screeners and senior staff were missing approximately 50% of all endometrial cancers, a thorough review of the sources of error was undertaken. It became evident that casual screening of vaginal smears is a poor tool for detection of endometrial cancer.

Therefore, especially careful screening procedures were applied to the following categories of patients:

1. Postmenopausal patients with evidence of abnormally high maturation of squamous cells.
2. Patients shedding endometrial cells, however normal appearing, outside of the physiological menstrual bleeding.
3. All patients in the age group of 45 years or over with either clinical or cytological evidence of abnormal bleeding.
4. All patients who have histiocytes in smears after the 12th day of the cycle.

Endometrial aspiration specimens have been of limited assistance. As a rule, when cancer is present it will be seen in the vaginal smear. This special care has rendered the results of screening for endometrial cancer considerably more accurate, culminating in detection of several early cases of endometrial cancer.

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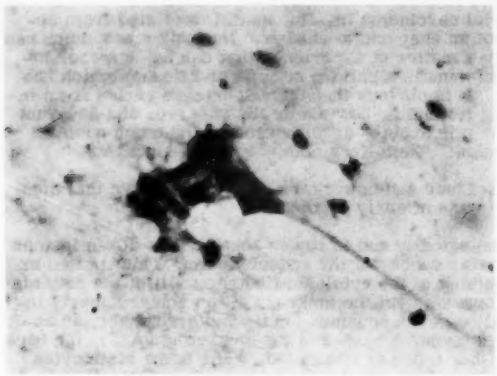

Fig. 1. Atypical endometrial cells in vaginal smear of 56 year old patient with carcinoma in situ of the endometrium (X440).

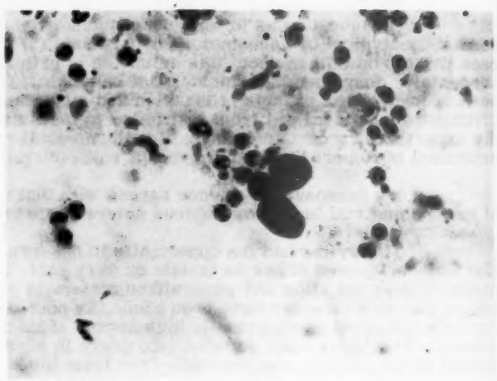

Fig. 2. Atypical endometrial cells characterized by increase in cell size with low nuclear-cytoplasmic ratio (X440).

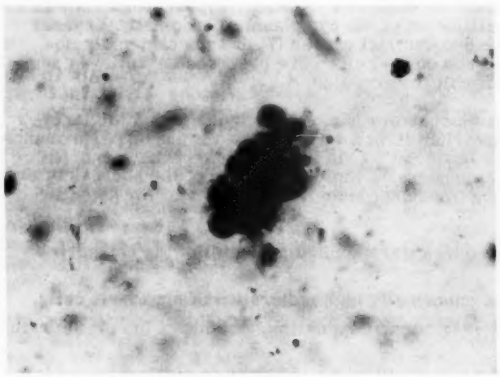

Fig. 3. Malignant endometrial cells, showing marked increase of nuclear-cytoplasmic ratio (X440).

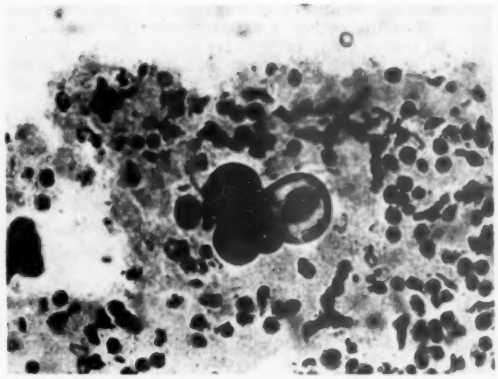

Fig. 4. Clump of malignant endometrial cells with one large pink endometrial cell from intra-uterine aspiration of 60 year old woman with carcinoma in situ of the endometrium (X440).

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LEOPOLD G. KOSS

New York, New York, U.S.A.

While the concept of "carcinoma in situ" of the endometrium may appear relatively new (1, 2), in reality the entity has been noted by Cullen (3) as far back as 1900 and was amply described by Meyer (4), who designated it by the simple name of "endometrial adenocarcinoma without evidence of invasion." I don't think that there has ever been a better definition of in-situ carcinoma than the one quoted above. In the case of the endometrium, the question of invasion is somewhat easier settled than in the case of the cervix because of the anatomical setting and a sharp demarkation between the glandular endometrium and the myometrium. To my mind, the presence of cytologically abnormal endometrial glands not surrounded by endometrial stroma within the uterine muscle constitutes evidence of invasion.

Fig. 1. Vaginal smear containing two enlarged endometrial cells, one of which has at least two nuclei. The cytoplasm of this cell is densely infiltrated with polymorphonuclear neutrophiles. The three smaller cells on the right side of the picture display multiple nucleoli.

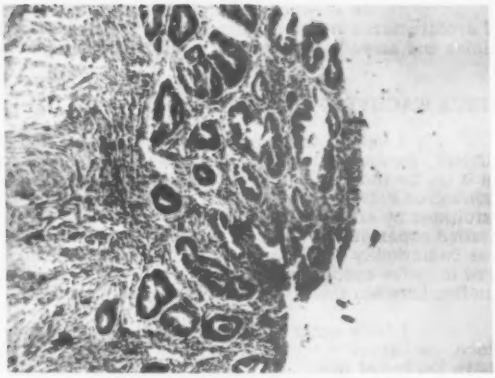

Fig. 2. The corresponding tissue section displays a highly abnormal endometrial pattern without any evidence of invasion.

DISCUSSION

ANTHONY F. ANDERSON, Edinburgh, Scotland, U.K.:

1. To von Haam: I should have been interested to know how soon after curettage his hysterectomy specimens showed no evidence of neoplasm, for it is not uncommon, as Novak maintained, for this to happen with a report of acknowledged adenocarcinoma – let alone an in-situ lesion. I agree entirely with his statements and have no further comment except that such a conclusion could be predicted by the pathologist, because curettings showing acknowledged adenocarcinoma from the uterine cavity are by their nature "in situ," though few seem to realize it. Hertig's work is an attempt to spot adenocarcinoma before it reaches this picture which we all recognize.

2. To Koss: I am grateful for Robert Meyer's designation which, as I have maintained above, we should really apply to all our reports on curettings. (If myometrium is seen in these, the curette is being wielded more deeply than most of us would approve of.) I am grateful for Koss' own evidence of invasion, but I feel he should emphasize "within the uterine muscle" as well as "cytologically abnormal," and glands "not surrounded by endometrial stroma." In his last sentence before his listed categories of patients, I trust he meant "routine" screening, not "casual." Finally, the fact that he gives no description of the cytological picture in carcinoma in situ almost implies that it is not possible. I would agree wholeheartedly.

JEAN de BRUX, Paris, France:

I have read very attentively the reports by von Haam and Koss. I agree with them that the cytological diagnosis of carcinoma in situ may be made by the following criteria:

- scarcity of abnormal elements;
- cellular atypias-relatively little marked;
- clean smears, with a high percentage of pyknosis and eosinophilia;
- occurrence in post-menopausal patients.

The difficulty stressed by the authors in making the cytological diagnosis is equally great when it concerns the pathological diagnosis, and depends on the pathologist's experience as well as on his school of thought.

It is certain that non-invasion is the first of the criteria, but this seems to us definitely insufficient. Many cases cases have been encountered of adenocarcinomas long present in the uterine cavity without invasion, but which could, nevertheless, not be considered as carcinomas in situ. Moreover, there exist very atypical hyperplasias which pose the problem of malignancy, concerning which Meyer advised: "Kein Karzinom, aber besser heraus." It is most regrettable that since that time no new light has been thrown on the problem of the differential diagnosis between the dystrophies and endometrial cancer. The recent symposium of the Royal Belgian Society of Gynecology and Obstetrics held at Brussels in July, 1958, brought no enlightenment.

In the absence of valid chemical or enzymatic criteria, we shall be obliged to agree on a system of architectural and morphological criteria which will be universally accepted, and furthermore to send slides and smears "around the world," asking specialists for the reasons for their particular diagnoses.

ERICA WACHTEL, London, England, U. K.:

I have nothing to add to the excellent and complete description by the two main speakers on the subject, except perhaps to issue a warning against further confusing the concept "in situ," controversial as it is, by the cytologists adding yet another definition. Von Haam speaks of a quantitative difference in exfoliation between invasive and non-invasive carcinoma and emphasizes that the morphology of the cells exfoliated by either type of lesion is identical. I can confirm this statement from my admittedly very limited experience with carcinoma in situ of the endometrium. The number of exfoliated malignant cells was remarkably small in all of these patients, but, on the other hand, I have also seen several cases of true invasive endometrial cancer with equally poor exfoliation and I, therefore, do not think that a distinction between these two conditions can be made on a cytological basis.

I fully agree with Koss about the necessity of a special search for endometrial cancer cells, since, on casual screening, they are more liable to escape notice than malignant squamous cells and I share his belief that cytology is an excellent method for earlier recognition of endometrial malignancies. However, the extent of the lesion on the surface as well as in depth can only be determined by histological examination of the curettings as well as the hysterectomy specimen.

Last, I would like to discuss an unusual case of endometrial carcinoma detected by routine cytology in a young asymptomatic woman:

The 30 year old married patient had no complaints except of one attack of painful menstruation following the admission of her husband to a T. B. sanitorium. Her smears contained a few endometrial cancer cells (Fig. 1) and subsequent curettage confirmed the diagnosis. She was re-admitted 3 weeks later for hysterectomy when repeated vaginal smears not only failed to show continued evidence of malignant disease but appeared to be completely normal Class I smears. Nevertheless, hysterectomy was performed in view of the previous findings but no remaining pathology was present in the removed uterus. In retrospect, we came to the conclusion that it would have been wiser to have repeated the curettage in view of the negative smear findings than to deprive that young woman of her uterus.

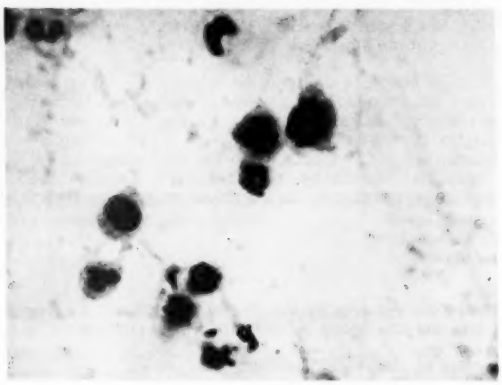

Fig. 1

CLOSING REMARKS

EMMERICH von HAAM:

In answer to the question of Anderson, I may state that patients are usually re-examined not earlier than two weeks after a cervical biopsy or four weeks after a curettage. De Brux refers in his remarks to the malignant endometrial polyp, which shows no invasion of the myometrial wall but otherwise shows all characteristics of carcinoma. I agree with Erica Wachtel that the uterus could have been saved in her case by a second curettage, which was definitely indicated by the negative cytological findings.

LEOPOLD G. KOSS:

I have but one question for de Brux. How does he classify adenocarcinomas within the uterine cavity without invasion since apparently he does not consider them as carcinoma in situ?

Cases such as the one reported by Erica Wachtel, in which carcinoma was found on curettage only, are not too uncommon. Usually a careful search by multiple sections of the endometrium will reveal a focus of endometrial atypia. Not to remove such uteri would be poor medicine.

CYTOLOGY OF UTERINE SARCOMA AND CHORIONEPITHELIOMA

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

Though sarcomas often exhibit elongated forms in the histologic picture, such elongated forms have not been encountered in the desquamated cells. In the eight cases of sarcoma of the uterus which we have seen, all have had only small undifferentiated cells - sometimes occurring as free malignant nuclei and occasionally having indefinite tags of cytoplasm.

We have made fresh preparations of the surgically removed sarcomas and in these the malignant cells are very bizarre and elongated in shape. It is peculiar that we have not seen such typical malignant cells desquamating. Of course, in many instances the sarcoma of the uterus will not desquamate any malignant cells since the tumor does not involve the surface of the endometrium. It may be that when malignant cells are found we are dealing with a far advanced tumor, and that in such tumors the majority of the malignant cells have lost their differentiation.

There have been reports of finding very atypical, large malignant cells in chorionepithelioma. This has not been our experience, but we have found only undifferentiated tumor cells, and cytologically could not have distinguished the cells from any other undifferentiated tumor. One of these cases is of interest, because the patient had a vaginal metastasis and a fairly large number of cells could be found in the vaginal smear. She was treated with methotextrate. No specific change was seen in the tumor cells under the influence of the drug. However, three months after the beginning of treatment, no tumor cells were present and her vaginal smear was completely normal and has remained so.

VIOLETTE M. NUOVO
Paris, France

I. SARCOMA

Sarcoma is a rare entity. In eight years, among 32,333 gynecological patients, 4 cases of sarcoma of the uterus have been diagnosed. We shall summarize the history of these four patients.

Case 1. A 52 year old woman came to the clinic for post-castration bleeding. At the age of 47, she was castrated by X-ray and had received androgen therapy for a fibroid tumor. Pelvic examination revealed an enlarged uterus. Malignant cells were identified on the vaginal smears. The origin of these cells was not given. A hysterectomy was performed and the pathological diagnosis was sarcoma.

Case 2. An 83 year old patient came to the clinic complaining of a bloody vaginal discharge. She had had a sub-total hysterectomy 2 years before. Pelvic examination revealed a cervix with an open os and profuse bleeding. Vaginal smears were classified as Class III because of fibroid cells with irregular nuclei. The repeated smears were classified as Class V because of large elongated and granular free nuclei. The biopsy showed a sarcoma (Fig. 1).

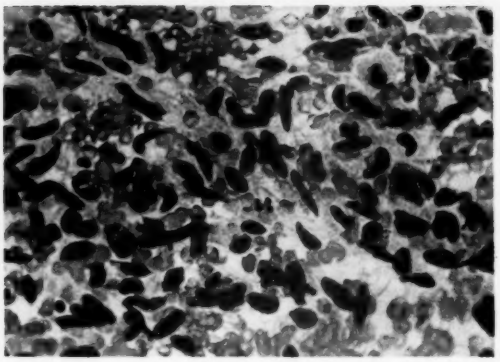

Fig. 1

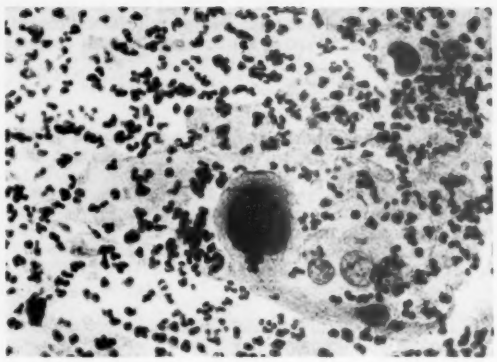

Fig. 2

Case 3. A 63 year old patient came to the clinic because of leukorrhea. The vaginal smears were typed as Class III, and when repeated, as Class IV because of small round cells with big irregular hyperchromatic nuclei. The cytological diagnosis was atypical glandular carcinoma. The pathological report on the operative specimen was a sarcoma (Fig. 2).

Case 4. A 28 year old patient was hospitalized for vaginal bleeding. She had had some "metrorrhagia" for 4 years. At the time of the pelvic examination, a painful mass was felt. There was no cytological examination at that time. An operation was performed, and smears were done on the operative specimen. They showed elongated granular nuclei. The histological examination showed sarcoma.

Cytology. In 3 cases, malignant cells could be found in the vaginal smears but they never were identified as sarcoma cells. In 2 cases the cytological report revealed that the type of malignant tumor could not be identified (Case 1 and 2). In one case (Case 1) the cells found were huge, round and swollen. In the other (Case 2) the neoplastic cells and nuclei were elongated. In 1 case (Case 3) the cytological report was atypical glandular carcinoma.

In the fourth case, no vaginal smears were available, and the cytological report of a specimen taken in the endometrial cavity during the operation pointed out elongated large granular free nuclei as in Case 2.

Thus, there seems to be a variety in the types of malignant cells found in cases of sarcoma, and we have only been able to diagnose cytologically the presence of malignancy without being able to classify it.

II. CHORIONEPITHELIOMA

Being interested in the chorionic epithelioma problem, we first examined vaginal smears of 35 cases with hydatiform mole. Nine cases had smears before delivery only. All of these smears were negative. Three cases had smears before and after delivery. All smears were negative before delivery. After delivery, one was still negative and two were typed as Class III. In one case, the smears were taken the very day of delivery and were reported negative when repeated. In the other case, the smears were taken 3 months after delivery and were reported negative when repeated. Twenty-three cases had smears only after delivery. Twenty-two cases had negative smears. One case had suspicious smears two weeks after delivery and was reported negative when repeated.

Apart from this study, there were 8 cases of probable chorionic epithelioma (See Chart). Among these 8 cases, seven had vaginal smears before operation; three were positive for chorionic epithelioma, one was suspicious as was the pathological report on the operative specimen and two were negative. In both cases the pathological report was positive for chorionic epithelioma, but one of these cases is still alive and in good health five years after treatment. One had poor smears and one had no smears before the operation but had positive smears on the operative specimen.

In all positive smears we found the same type of atypical cells: large cells with huge nuclei which were irregular and granular with enlarged nucleoli. It seems that the diagnosis is as difficult to obtain both pathologically and cytologically, since cytologically, the cells found in cases of chorionic epithelioma are very similar to the trophoblastic cells sometimes found normally during or immediately following delivery or abortion. In cases previously described, however, the nuclei were definitely monstrous.

Case No.	Obstetrical History	Symptoms	Vaginal Smears	Sputum Smears	Endo-metrial	Prolan U in Urine	Surgery & Histology	Cytology
1	hydatiform mole, delivery	hemorrhage 20 days after	Class IV; cells resembling trophoblastic cells			200 to 500	hysterectomy; chorio-carcinoma	
2	3 normal deliveries	mass observed in chest X-ray	Class IV	Class III atypical cells			hysterectomy; chorio-carcinoma (not sure). Lobec-tomy: chorio-carcinoma	Class V
3	hydatiform mole, delivery	none	Class IV		decidual benign polyp		hysterectomy; chorio-carcinoma	Class IV
4	hydatiform mole, abortion, curettage	Langhans' cells found	Class III			100,000	hysterectomy; chorio-carcinoma	
5	hydatiform mole, abortion, curettage	amenorrhea	negative	Class III			hysterectomy; chorio-carcinoma	
6	hydatiform mole, abortion, curettage	none	no smears taken			10,000 to 50,000	hysterectomy; chorio-carcinoma	
7	hydatiform mole, abortion	enlarged uterus 3 weeks after abortion. Chest X-ray normal	Class II Class III on repeat smear		negative	1000	hysterectomy; borderline case: hydati-form mole -- chorio-carcinoma	
8	2 normal deliveries, hydatiform mole 7 years before detec-tion of chest lesion	mass detected in chest X-ray	negative			1000 to 10,000	hysterectomy; chorio-carcinoma. lobectomy: chorio-carcinoma	

ANDRÉ RAUZY and JEAN A. de BRUX
Paris, France

Chorionepithelioma and sarcoma of the uterus only have rarity and malignancy in common (2 to 3 percent of all malignant tumors of the reproductive system). They are different in origin - the sarcoma being an autochthon tumor while the chorionepithelioma is an implantation tumor.

Arising from the adult uterine tissue and having regained the neoplastic potentialities of the embryonal life, the sarcoma undergoes for a long time a local and then a regional development. It is not hormone-producing. It will rest for a certain period of the time, curable by a local therapy.

Originating from the trophoblast of an ovum, mostly a mole, the chorionepithelioma is hemorrhagic: It perforates the vascular septa and penetrates into the blood stream, thus voiding the effectivity of all local therapeutic measures. As a highly hormonally active tissue it interferes with hormonal balance, which already is disturbed by the pregnancy, this explains its humoral aspects and the histological patterns of the hormonal receptors.

These great differences of the two tumors, thus, require that they cannot be treated within the same paper. That is why we shall consider only the chorionepithelioma. The problem posed by the chorionepithelioma is extremely difficult. Its clinical aspects are extremely manifold: hemorrhages, metastases, elevated or increasing prolanuria, invasion by the trophoblast, which is anaplastic and hyperplastic.

But none of these criteria is absolute and neither the clinician, the endocrinologist nor the histologist are capable of differentiating a dissecant mole treatable by local means from a chorionepithelioma - the only criterion for malignancy being the death of the patient.

The Role of Cytology in Detection, Diagnosis and Prognosis of Hydatiform Moles and Chorionepithelioma:

Its insufficiency has several causes:

1) Small number of cases: One mole in 1000 to 2000 pregnancies and one chorionepithelioma in 10 moles. Moreover, the smears are somewhat neglected in the follow-up of abortion cases, even though we are dealing with moles.

2) The poorness of certain vaginal and cervical smears on characteristic elements: In contrast, the endometrial smears are rich and exemplary, like imprints of the tumor itself. These endometrial smears are easy to obtain and without danger.

3) The eccentric site of the proliferative growth, with its nuclei deeply invaded into the myometrium and into the vessels. Moreover, being separated from the uterine cavity by a thick layer of fibrin, blood and inflammatory elements isolates and submits them to profound degenerative changes.

4) Besides all these factors, the cyto-diagnosis is difficult in itself.

On the smears, three different types of morphological elements (clusters, free nuclei, isolated cells) are very often encountered in an environment which is very frequently strongly hemorrhagic and leukocytic.

The Clusters:

They differ in size and gather in groups of 10 to 40 nuclei which are slender and some times even tadpole-shaped. The cytoplasm is fine, lightly cyanophilic or polychromatic, with fine vacuoles. It rarely contains inclusions, the edges are indistinct, fringed, and one can observe the cells beginning to separate from each other. The cellular borders are not very sharp. They are, at best, marked by a brighter line which surrounds the cytoplasm and adjacent nuclei. At other places the appearance becomes syncytial. They present quite a diversity in their nuclear structure:

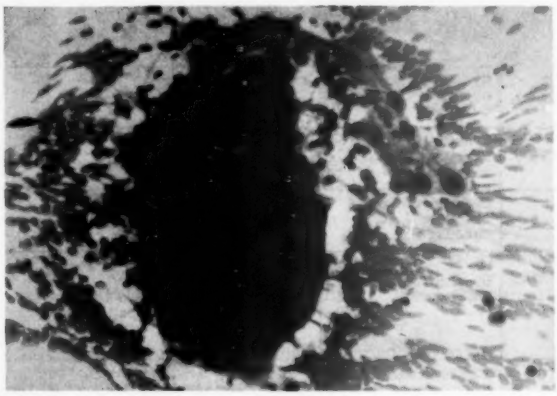

Fig. 1

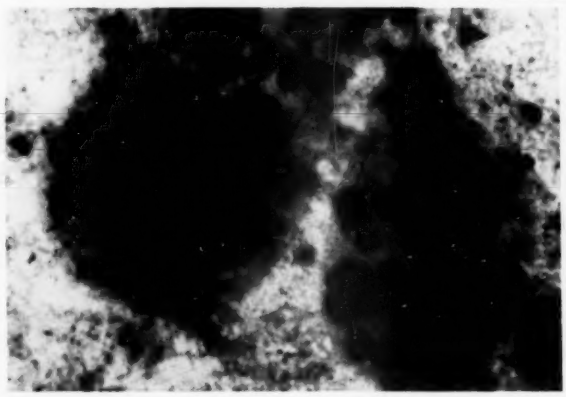

Fig. 2

Some have small nuclei (4 to 5 μ), round or oval shaped, regular, clear with even chromatin distribution, and 1 or 2 nucleoli. They are sometimes very crowded and have a tendency to overlap each other. Their similarity to certain endometrial clusters is surprising. We can observe the little rounded clusters with small concentrically placed nuclei, which are very much like some forms of hyperplasia.

However, some among them have peculiar chromatin distribution - point-like, with great regular grains and a clear membrane (Figs. 1, 2).

The other clusters do not display this regular aspect and the predominating impression is that of anisokaryosis. In fact, side by side with the described nuclei, nuclei which are still clear, regular and oval-shaped but much larger are found: 10 to 15 μ in diameter, grayish, marked by their unique double nucleoli, very finely structured and of a puffy appearance (Fig. 3).

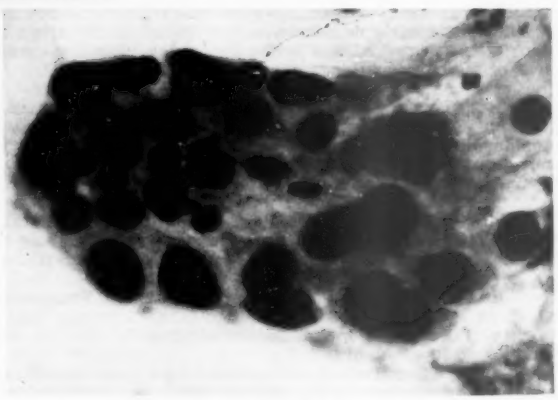

Fig. 3

Rarely one may observe very small clusters, with 4 to 8 nuclei together, which are very cytolyzed and the nuclei of which are frankly abnormal: voluminous (10 to 15 μ in diameter) polygonal and oval-shaped and unequal, prolonged with very slightly irregular borders. They appear hyperchromatic, sometimes however, the chromatin pattern is invisible, displaying only a uniform mass. Moreover, it presents irregularities, whirls, thick stripes or non-uniformly distributed masses, and it contains several nucleoli (Fig. 4).

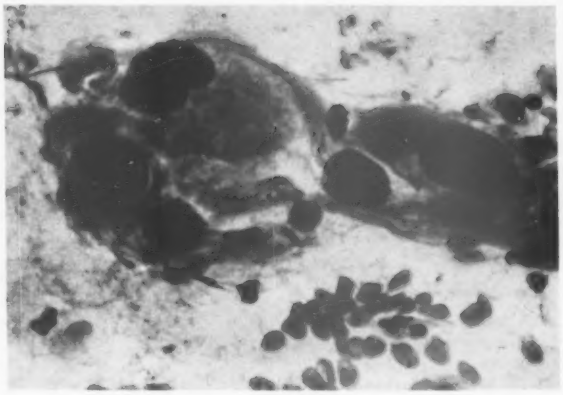

Fig. 4

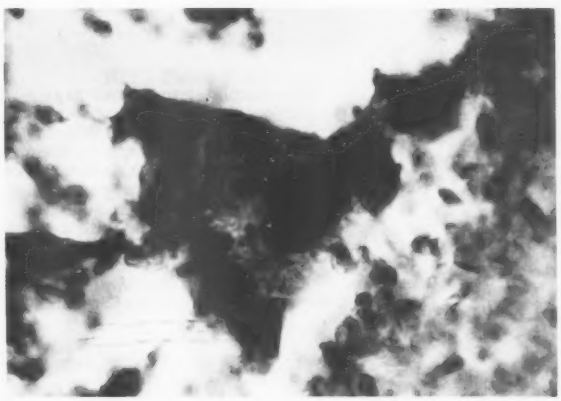

Fig. 5

The Free Nuclei:

They are very voluminous, mainly in certain endometrial smears. One type is very evident, e.g., the ones we have described as big, bright, voluminous and very hyperchromatic. They are found at the side of the clusters or also isolated, and they are very easily distinguishable from the background which consists of blood and mucus (Fig. 5).

The others demand more attention in order to be recognized. They have small, clear nuclei sometimes kidney-shaped with turbulent chromatin, resembling the shape of histiocytes. Finally, plentiful masses of nuclear debris from the shady background in many slides.

The Isolated Cells:

The most unusual and apparently the most characteristic cells are large ones of 20 to 25 μ in diameter, of polygonal shape, sometimes irregular and bizarre, with occasionally fringed edges, the cytoplasm polychromatric and very finely vacuolized. Their nuclei are voluminous, thus inverting the nuclear-cytoplasmic ratio. They are also hyperchromatic. The same ones we have described under "clusters." Thus, sometimes they show irregularities in their design and chromatic structure. Sometimes these characteristics are vague because of poor staining properties. It appears as a soft purplish mass and sometimes these cells are multinucleated (Figs. 6, 7).

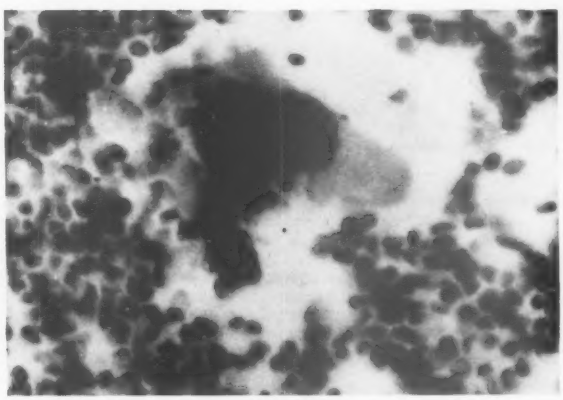

Fig. 6

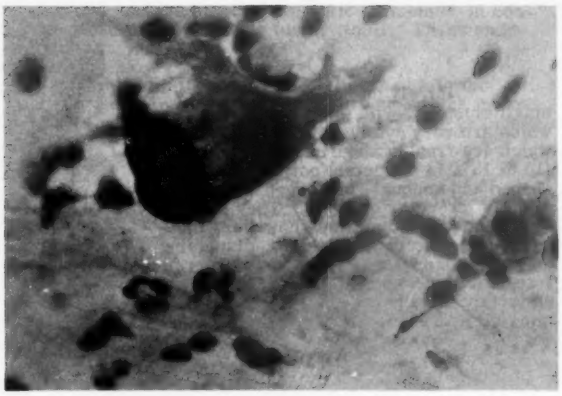

Fig. 7

Cells Interpretation Difficulties:

One also encounters cells with an ordinary appearance, faintly cyanophilic, finely vacuolated, with normal nuclei and normal nuclear-cytoplasmic ratio, resembling decidual cells (Fig. 8).

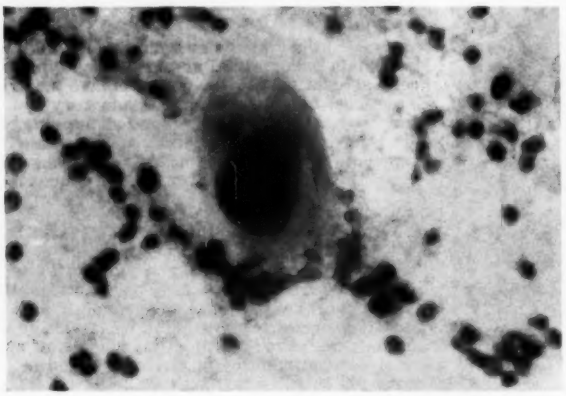

Fig. 8

Other cells, sometimes in groups of 3 or 4, present degenerative phenomena, either large cytoplasmic vacuoles which push away the nucleus or rarely some inclusions (leukocytes) (Fig. 9) or nuclear changes: under the picture of retraction they become small with rugged edges, with opaque and irregular contents, sometimes with a clearer cytoplasmic zone surrounding them. But this is only the beginning of a pyknotic process which eventually is completed and releases the nuclear debris already mentioned (Fig. 10).

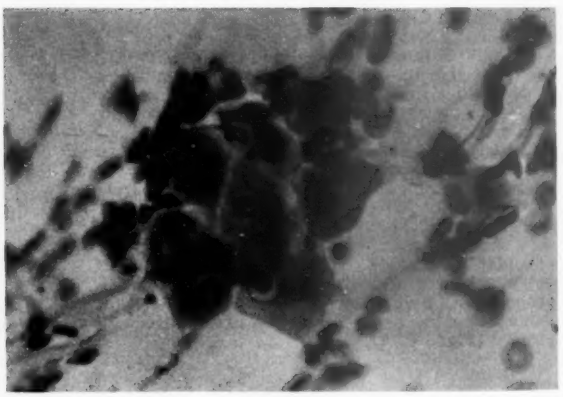

Fig. 9

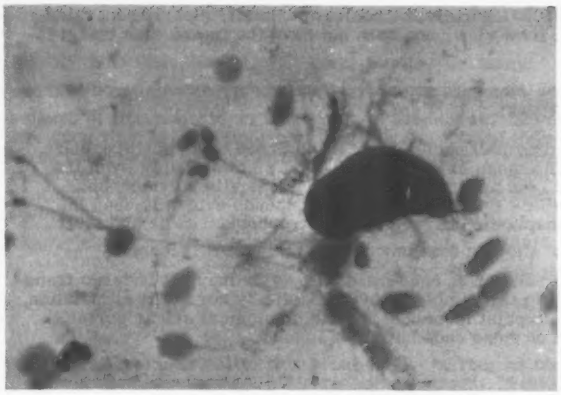

Fig. 10

We have examined the imprints of the chorionepithelioma and the corresponding histological sections.

A. The imprints of chorionepithelioma show two major cell types:

1. Polygonal irregular cells with dark nuclei
2. Clusters which are often small and round, with regular nuclei, coarsely grained chromatin and a clear membrane

B. These pictures correspond very well to those which are found in the histological sections. As a matter of fact, the chorionepithelioma displays two different kinds of cells (Fig. 11):

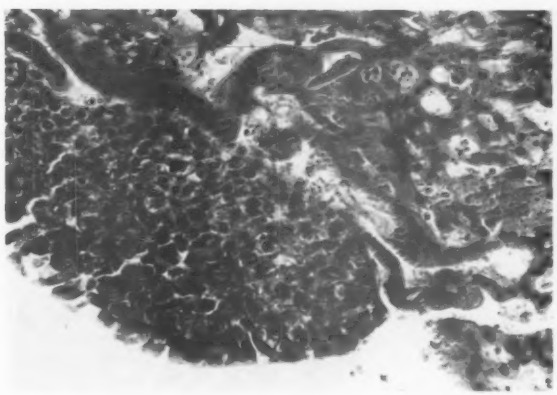

Fig. 11

1. The cytotrophoblast
2. The synctiotrophoblast

1. The cytotrophoblast sometimes appears as those large clusters, the single elements of which are fairly regular looking with more or less vacuolized cells and rather large nuclei. Yet there is the impression of uniformity and the idea of malignancy is not evident from first view.

   But aside from these, one discovers clusters with very anaplastic behavior. These cells are much larger, with big, unequal, hyperchromatic nuclei, prominent nucleoli and avacuolized cytoplasm.

   On the rim of these clusters, sometimes very large elements, the nuclei of which occupy almost the whole cell, are seen. Their nuclear borders are faint, the chromatin pattern is turbulent, and sometimes it is clearly reticular and sometimes it is not visible at all. The cytoplasm is cyanophilic. These are the injured cells.

2. The syncytiotrophoblast is a more or less extended plasmodium, often of elongated shape. The cytoplasm is eosinophilic and perforated by large vac-

uoles, forming spaces. Generally the nuclei are small, clearly separated from each other. On first view, one does not have the impression that it is malignant tissue.

The correlations of the elements as observed on smears as compared to those on sections are as follows:

a. The clusters with small nuclei correspond to the syncytiotrophoblast.
b. The clusters with more or less large nuclei stem probably from the cytotrophoblast, the appearance of which is actually polymorphic.
c. The isolated cells: Their polymorphism depends evidently upon the more or less anaplastic character of the trophoblast cells and their degree of necrosis.

Where the endometrium is concerned, it may or may not undergo transformation. It also can simply be hyperplastic by hormonal stimulation, and neither the imprint technique nor the cytological smear permits our recognition of these endometrial elements.

Follow-Up of the Patient:

The diagnostic difficulties of chorionepithelioma and the fact that it disguises itself as a complication of a hydatiform mole require repeated examinations and thorough follow-up of molar abortions. Besides checking for the presence of trophoblastic elements, it is convenient to determine the hormonal status because they are hormone producing.

We could, in one case, state hormonal activity from the smear without encountering trophoblastic cells, and these did not appear until 3 weeks later. Thus, it should be possible to calculate the ratio between the appearance of the smear and the "biological" hormone dosages.

Yet cytology can only detect the trophoblastic elements on the smear without being able to differentiate between benign and malignant. If any differential criterion exists, it will be, without a doubt, cytochemical and not morphological. This same conclusion is revealed by the clinical experience, by the endocrinology and by the history as well. The cytological smears have their well established place among those techniques which help the practitioner, who is constantly challenged by the heavy responsibility for the diagnosis and for the therapy as well.

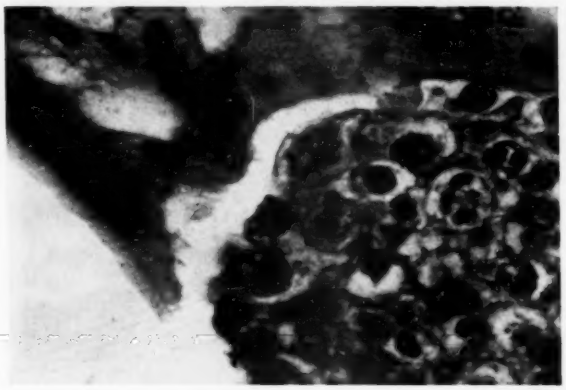

Fig. 12. Choriocarcinoma: trophoblastic cell proliferation, and syncytiotrophoblasts.

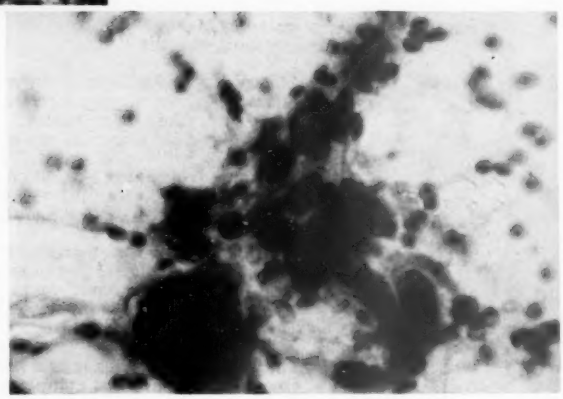

Fig. 13. Smear of choriocarcinoma: A large trophoblastic cell and several syncytiotrophoblastic cells.

DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

During the last five years, we have had the opportunity to see the vaginal and endometrial smears of five patients with sarcoma of the uterus. All of the smears were judged as positive (four or five according to Papanicolaou's technique). Two of them were suspicious for sarcoma, while the other three cases could not be distinguished from de-differentiated carcinoma. Only these three cases showed the picture described by Ruth Graham: small undifferentiated cells, sometimes occuring as free malignant nuclei and occasionally having indefinite tags of cytoplasm. In addition, the other two cases manifested the following:

One case: Coherent clusters of stroma cells with marked nuclear hyperchromasia and irregularity in shape and size, and many multinucleated giant cells.

One case: Many strips of elongated spindle-shaped stromal cells, different from spindle-shaped squamous cells.

Cytometrically, this group of five sarcomas could not be differentiated from the group of undifferentiated carcinomas. The reason apparently is the preponderance of the undifferentiated cells described by Ruth Graham. Similar multinucleated giant cells were found in the ascitic fluid of an ectopic chorionepithelioma of the liver in a 67 year old patient manifesting a luteal phase in the vaginal smear and decidual endometrial stroma cells in the aspiration smear from the uterine cavity.

Agreeing completely with Violette Nuovo, we consider it very difficult, or completely impossible, to differentiate cells of a chorionepithelioma from trophoblastic cells which are shed following delivery or abortion.

I would like to ask Ruth Graham and Violette Nuovo about the hormonal pattern of their patients with chorionepithelioma, since the production of hormones by this lesion has often been variously described.

MANUEL GALBIS, Valenica, Spain:

I wish only to make some remarks on the diagnosis of chorionepithelioma, the difficulties in the histological diagnosis of which are well known. In 1957, we undertook a histochemical research on 8 cases of hydatiform moles and one case of chorionepithelioma with the hope of finding some differences between malignant and non-malignant trophoblastic proliferation. We have investigated the following reactions: Feulgen reaction for DNA, methyl-green pyronin and cytoplasmic cyanophilia for RNA, Best's carmine and PAS reaction with salivary digestion for glycogen, PAS reaction and metachromasia with toluidin blue for mucopolysaccharides, and acid or alkaline phosphatase. The results were not conclusive and we could not find any significant differences between the vesicular mole and chorionepithelioma.

The above reactions were performed on smears obtained by means of vaginal and cervical aspiration in 5 cases of vesicular mole and two cases of chorionepithelioma. Smears were also performed at the time of surgery. Morphologically, the mole shows occasional undifferentiated cells similar to the ones found in the chorionepithelioma, however, the atypia is more marked in the latter. The histochemical reactions show a considerable irregularity in both instances and, as in the tissues, it is not possible to find any significant differences between mole and chorionepithelioma.

In both instances the glycogen is lightly distributed. The RNA is strongly positive without noticeable differences. The non-metachromatic mucopolysacchrides (glycoprotein?) offer marked reactions. The alkaline phosphatase, in spite of its irregular distribution, can be strongly positive in both instances. The acid phosphatase has been negative in our material. In contrast with the normal trophoblast, in malignant degeneration there is an increase of glycoprotein, RNA and alkaline phosphatase, but the same can be seen in the vesicular mole. In this respect, then, there is no definite pattern for determining the malignancy of a trophoblastic proliferation.

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F. A. IKLÉ, St. Gallen, Switzerland:

Besides the malignant chorionic neoplasms, i.e., chorionepitheliomas and the myogenic sarcomas, both of which are out of the scope of cytology, there are some rare sarcomas of the uterus, exfoliating highly malignant cells. These tumors arise from the mucosal stroma or from the connective tissue of the body of the uterus, cervix and vagina. They probably often belong to the so-called mesodermal mixed tumors of the female genital tract.

Among 9500 patients who routinely took smears for cancer detection, sarcoma was suspected in only three cases.

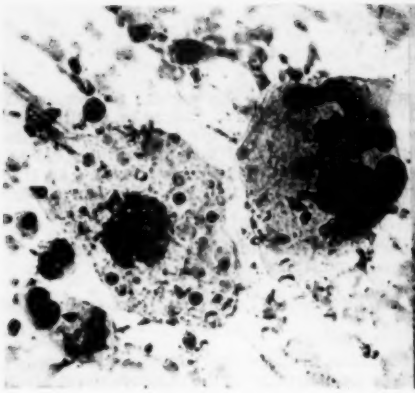

Fig. 1a

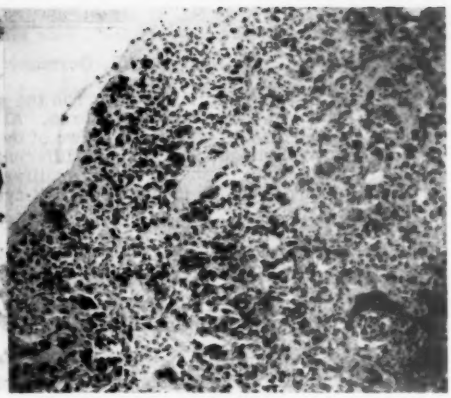

Fig. 1b

A woman, 63 years of age whose menopause had been artificially induced by external x-ray treatment at 40 years, had a D & C for recurrent uterine bleeding. No carcinoma could be detected histologically. One year later a vaginal smear was taken because there was still some uterine spotting. Due to the bizarre giant cells (Fig. 1a), a sarcoma of the uterus was suspected and was also histologically proven (Fig. 1b).

A woman, 44 years of age, had a subtotal hysterectomy for large fibromyomas of the uterus. One year later a smear scraped from the cervical stump showed atypical cells with dark hyperchromatic nuclei (Fig. 2). The stump was curetted, but no biopsy was taken. The histological findings were negative. After a delay of four months, a friable, bleeding, polypoid tumor, protruding from the cervix could be seen by the naked eye. Histologically, the tumor now proved to be a partly spindle-celled, partly round-celled sarcoma. It did not respond to radium and x-ray treatment. The woman died shortly afterwards from multiple metastases.

A woman of 41 showed a finger-sized cervical polyp, which had not been detected on a routine pelvic examination three months previously. Curettage revealed a friable, polypoidal growth protruding from the uterine cavity through the cervical os.

The curetted material was histologically interpreted as sarcoma of the endometrial stroma, while the gross and microscopic picture of the hysterectomy specimen was that of a so-called sarcoma botryoides, i. e., mesodermal mixed tumor. In the smear, spindle-shaped atypical cells with large, oval nuclei were suggestive for malignancy, but did not allow further differentiation (Fig. 3).

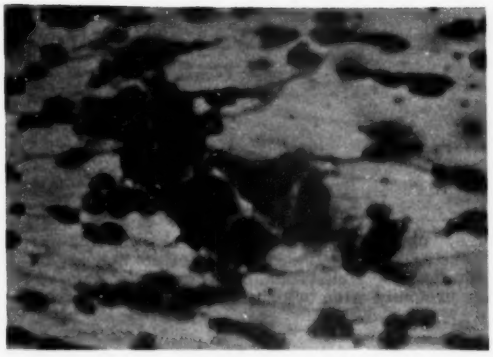

Fig. 2

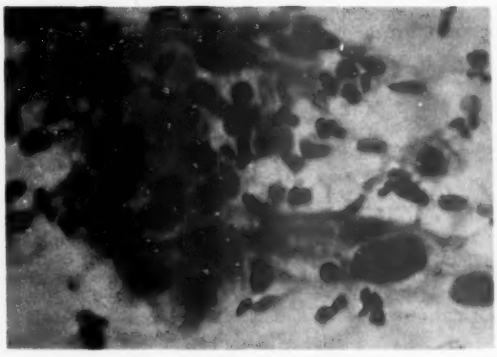

Fig. 3

CLOSING REMARKS

RUTH M. GRAHAM:

In answer to Boschann's question concerning the hormonal patterns, I am not sure whether or not he is referring to evidence of chorionic gonadotrophin or is questioning the cytologic evidence of any unusual hormone elaboration.

The benign squamous cells of the two patients with chorionepithelioma were not at all remarkable. One had 90% intermediate cells, 1% basal cells. In the second, the counts were as follows in percentage of intermediate cells: 71%, 84%, 74%, 74%, 80% over a period of three months. There were never more than 1% basal cells. The cytohormonal pattern did not change in any degree even after successful chemotherapy.

The chorionic gonadotrophin levels were low in each instance. In one case, 2500 mouse units and in the second, 1,000 + mouse units.

VIOLETTE M. NUOVO:

In response to Boschann's pertinent question concerning the hormonal pattern of the previously discussed cases of choriocarcinoma, our study revealed the following:

Case I. On the smear taken two weeks after delivery of a hydatiform mole, the Karyopyknotic Index was 88% and the Eosinophilic Index was 18%.

Case II. Five months after normal delivery, the Karyopyknotic Index was 88% and the Eosinophilic Index was 73%.

Case III. After a hydatiform mole delivery and on the 21st day of the cycle, the Karyopyknotic Index was 81% and the Eosinophilic Index was 69%.

Case IV. Two months after hydatiform mole delivery, the Karyopyknotic Index was 9% and the Eosinophilic Index 17%.

Case V. Because of a marked trichomonad infection, a proper hormonal picture was unavailable.

Case VI. No vaginal smears were obtained.

Case VII. Three months after the expulsion of a hydatiform mole, the Karyopyknotic Index was 34% and the Eosinophilic Index was 18%.

Case VIII. The occurrence of Trichomonas vaginalis prevented adequate interpretation of the smears.

It is important to note that of the 8 cases, 3 had a significantly high pyknotic level, expressed by a percentage of over 80%; one case was within normal range exhibiting a Karyopyknotic Index of 34%; and one case presented a low percentage of pyknosis. The three remaining cases were impossible to evaluate because of trichomonas infection and lack of smears.

ANDRÉ RAUZY:

We agree with the discussants.

According to the most recent knowledge of this subject, it seems difficult to differentiate many sarcomas from ordinary carcinomas, and of even greater importance - the benign mole from the chorionepithelioma. Even histochemical and cytometrical studies are of minor help.

Concerning the chorionepithelioma, the follow-up of the hormonal status on the smears is certainly interesting and might merit systematic studies.

Chorionepithelioma and sarcoma are certainly very infrequent uterine tumors, the rarity of which seriously complicates the further advance in knowledge, and at the same time, investigations are made difficult by the nature of the tumor itself.

Any procedure which permits cytologists to collect more cases, such as the pathologists did for chorionepithelioma, certainly shall be of great profit.

Symposium B

TECHNIQUES FOR ENDOMETRIAL CYTOLOGICAL EXAMINATIONS

ADVANTAGES AND DISADVANTAGES OF INTRA-UTERINE ASPIRATION TECHNIQUE FOR ENDOMETRIAL CYTOLOGY

PIERRE HAOUR
Lyon, France

An important point in obtaining cytological material is to try to collect cells as near as possible to the site of desquamation; this is the main advantage of the endometrial aspiration technique.

First, one may consider that endometrial cells are not always found in the cervical or vaginal pool. In the normal menstrual cycle they only appear during menstruation or during the post-menstrual stage. In the routine detection of cancer, we examine both vaginal and cervical smears; an appreciable number of slides do not show endometrial cells, and an intra-uterine aspiration is often necessary to detect adenocarcinoma. Another advantage of smears made with the material aspirated from the uterine cavity is that they are not contaminated with cells from the cervix or the vagina: endometrial cells are found exclusively, or at least predominately. With the aspiration technique, the cellular structure also, will be better preserved. Endometrial cells appear in clusters and the aspiration tube may even bring back small pieces of epithelium which can be mounted or sectioned to be compared later with the smears. Cyto-chemical procedures can be applied to this material, and are more effective than when degenerative changes affect the cell structure; this is often observed in vaginal smears and can induce misinterpretation, as has been noted by others (1).

However, intra-uterine aspiration requires a number of operations: insertion of a speculum, cleansing and disinfection of the cervix; sometimes it is necessary to use a tenaculum to introduce the tube through the endocervix. Care should be taken also in the preparation of smears: anytime there is a lesion of the endometrium, a hemorrhage may easily be induced by the trauma of the aspiration technique. The smear then will contain very few cells or be entirely made up of blood. This disadvantage may be corrected if one follows the centrifugation technique in preparing the smears. The method we use consists of thoroughly mixing the aspirated material with an equal part of saline; after centrifugation, the sediment is washed again with saline and centrifuged once more; the sediment is smeared and then we use the

collodion technique proposed for urine cytology (2). Several times we obtained cancer cells in the centrifugation smear, though the direct endometrial smear, as well as the cervical and vaginal smears, were negative.

Endometrial aspiration does not, therefore, appear as simple as other cytological techniques, and there may be some difficulty using it as a routine procedure. However, to those who want to properly study nucleo-cytoplasmic structures, as is often necessary to identify hormonal changes in endometrial smears (3) (4), or to make a diagnosis of cancer, this technique is justified. As it appears from the work of cytologists who could compare different procedures of cell collections (5) (6), endometrial aspiration evidently improved the detection of adenocarcinoma. In the hands of the gynecologist it is, like endometrial biopsy, a very simple procedure which is worthwhile to perform anytime the vaginal and cervical smears are negative and the clinical conditions require more investigation.

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B. CORNELIS HOPMAN

Miami, Florida, U.S.A.

The endometrial smear which is obtained by aspiration of mucus from the endometrial cavity by means of a cannula to which a syringe is attached, has distinct advantages and disadvantages.

The disadvantages are obvious: It is time-consuming, requires asepsis and must be performed by a physician, in contrast to the vaginal smear which can be done by a nurse or even by the patient herself (using a tampon, spatula or finger). The endometrial aspiration technique has certain dangers. Every instrumentation of the cavum uteri has risks of infection, trauma, even performation, although in the case of an endometrial aspiration these latter risks are almost negligible in the hands of a gynecologist.

The advantages surpass the disadvantages: the cytologic diagnosis of adenocarcinoma of the endometrium using vaginal and cervical smear techniques will be inaccurate in about 50 per cent of the cases with malignancy, in contrast to the very high accuracy of the cytological cervical cancer diagnosis. Since endometrial cancer occurs mostly in women during their postmenopausal years (when some degree of cervical stenosis has developed), exfoliated cells from the cavum uteri may not reach the cervix or may take so much time to arrive there that they are too degenerated for recognition. Furthermore, the intermixture of red and white blood cells makes identification still more difficult. It may be difficult to differentiate endocervical from endometrial cells. Endocervical cells are larger than endometrial cells. They show a distinct honeycomb appearance in transverse section and a columnar arrangement in longitudinal section. The cell border is more distinct and mucus or cilia protrude from their tops. Endometrial cells are smaller, show closer proximations, but isolated cells may be difficult to differentiate. Furthermore, in hyperplasia mucosae uteri, endometrial cells may be considerable larger. It may be difficult to differentiate endometrial cancer cells from the small undifferentiated squamous cancer cells as one encounters them in advanced cervical cancers.

The use of the endometrial aspiration technique obviates these difficulties because the source of the cells is beyond doubt. The cannula used in aspiration technique will often be able to pass a cervical stenosis and produce fresh cells, readily recognizable. It will eliminate confusion in identifying endometrial cells from either endocervical or undifferentiated squamous cells. There may still be squamous cells on the slides caused by passing of the cannula along the cervical canal, but usually these cells present no diagnostic problems.

Using endometrial aspiration smears one may be able to diagnose endometrial cancers which were missed by curettage.

GUILLERMO TERZANO

Buenos Aires, Argentina

The endometrial aspiration smear containing abundant endometrial cells and endometrial clusters gives more possibilities for a correct diagnosis if it does not contain mucus of the cervical canal. Endometrial smears can be taken even if the patient has had a recent vaginal douche.

Well-preserved endometrial cancer cells are infrequently found in the vaginal smears of a postmenopausal woman with early endometrial carcinoma compared to endometrial cells found in the vaginal smears of a normally menstruating woman between her menstrual periods. This is the principal reason (regarding cytodiagnosis of early endometrial carcinoma) for false negative reports in most of the available statistics from different laboratories. Therefore, the development of the endometrial smear technique was a welcome addition to our previous methods. The specimens directly obtained from the uterine cavity submit more adequate material.

Similar to the experience of Wied (Proc. Second National Cancer Conference, p. 1202) we found in 12 of our recently proven carcinomas of the endometrium:

positive vaginal smears in 5 out of 12,

positive cervical smears in 8 out of 12,

positive endometrial smears in 10 out of 12.

This is why we think there are only advantages using the endometrial aspiration technique in cytological cancer diagnosis. If a thin malleable cannula, with a small tip, is gently introduced through the cervical canal, the procedure is absolutely painless. Dilatation of the cervical canal or the internal os, is seldom needed.

Endometrial aspiration requires a physician to prepare the specimen, and the procedure takes time. Also the grasp of the cervix (in the few cases in which it is necessary) may cause some discomfort to the patient.

Vaginal infections, cervical infections or other inflammatory reactions as well as pregnancy, are formal contraindications of the introduction of a cannula into the uterine cavity. Also, when there is stenosis of the cervical canal, a cannula cannot be introduced. "Contraindication or impossibility" is, however, not the same as "disadvantage."

Although the endometrial aspiration technique cannot be considered a routine procedure, it has only advantages when used in the presence of any clinical suspicion of an early carcinoma of the endometrium (bleeding, bloody discharge, etc.), and especially in those cases in which the cervix looks healthy and the previous vaginal and cervical smears have been reported as negative.

DISCUSSION

GIUSEPPE DELLEPIANE, Torino, Italy:

There is no doubt that the technique of intra-uterine aspiration in the diagnosis of endometrial carcinoma, particularly that of the body of the uterus, gives better results than the vaginal smear. Therefore, one can agree with the above authors.

It does not seem to us, however, that the approach to the problem is correct, inasmuch as what is attainable with the vaginal smear method cannot be achieved with aspiration.

The vaginal smear is indicated in the mass screening procedure and also when there are lack of symptoms or doubts. The aspiration technique cannot be systemically applied because of the conditions of distress to which the patient is put and for the assistance that is necessary in such a situation; therefore, it is justified only if the suspicion of an endometrial neoplasm exists.

The correct approach to the problem should consider:

1. How is it possible to improve the results of the vaginal smear, as a mass screening for cancer, that is, in case of lack of symptoms?
2. What are the advantages and disadvantages of the aspiration technique, not in relation to the vaginal smear, but as referred to other histological or cytological endometrial techniques (curettage, washings of the uterine cavity, etc.)?

If this is the approach, then it seems to be logical that there are means for improving the results of the vaginal smear method, besides the endometrial procedures. The simplest of these means is the mechanical squeezing of the various uterine segments (Beclere maneuver for the endocervix) particularly the body of the uterus by means of the bimanual maneuver. The technique applied by Vecchietti (1) seems more rational, however, and we have been using it for quite some time. It consists of the intravenous injection of ergotamin tartrate (Methergin) followed by a vaginal smear. Such technique undoubtedly improves the results to such a degree as to deserve an extensive application, particularly where an instrumental procedure is not justified.

As to the second question, there is no doubt that the aspiration technique has advantages in comparison with other techniques of endometrial investigation (washing, curettage). Some objection could be presented, relative to the amount of blood that is obtained in this way. We do not think this to be so important, since with the technique we employ (karyological method) the treatment of the material with acetic acid takes care of that. With this method, since the cytoplasm is completely destroyed, we get a good enrichment of the cellular material, in particular the endometrial material. The blood elements are also destroyed by the acid (2).

Anyway, in comparison with curettage, aspiration proves to be inferior, for, if the aspiration is negative and the functional symptoms of carcinoma are present (hemorrhage), curettage cannot be avoided.

In any case, the advantages of aspiration over curettage appear very important (less trauma, less chance of infection and spread of cancer cells) and the former method deserves absolute preference whenever the vaginal smear is negative.

Furthermore, the positiveness of the smear does not exclude the biopsy (carcinoma of the cervix), nor the aspiration (endometrial carcinoma) before an operation can be planned, whereas a positive aspiration can be enough to justify treatment.

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MANUEL GALBIS, Valencia, Spain:

In our experience, the cytological diagnosis of adenocarcinoma of the endometrium has a high percentage of errors with the use of the vaginal smear. The problem is two-fold: First, there has to be malignant cells on the smear, and second, we have to be able to recognize them. Both premises present difficulties in the vaginal smear. The presence of endometrial cells is only occasional, resulting in an increase in the number of false-negatives. We have seldom made a diagnosis of adenocarcinoma in a woman without symptoms by the use of vaginal smears, and even in suspicious cases our errors are high (44.1%). Haour and Terzano found analogous difficulties.

The reliability of the procedure is greatly increased by the use of endometrial smears obtained by direct aspiration. This way we consistently obtained better preserved endometrial cells and a larger number of cell clusters. We fully agree with Terzano and Haour that direct aspiration has great advantages. However, the procedure is not a simple one for routine use and errors are not infrequent. We believe it has limited value for early diagnosis. In cases with clinical symptoms the diagnosis must rely on the histological study.

F. A. IKLÉ, St. Gallen, Switzerland:

The intra-uterine aspirations are certainly more difficult to perform than simple vaginal smears or cervical scrapings with the Ayre spatula. For the gynecologist or experienced practitioner, however, this technique does not offer any problems. The few contraindications are a question of common sense. There are very few complications. If handled gently, there is little pain. From 374 patients with ambulatory performed aspirations, only two showed asymptomatic fever on the next day. Thin, malleable cannulas of the nasopharyngeal type proved better than plastic polyethylene tubes or "endometrial brushes." The smears are fixed immediately and stained according to Papanicolaou's technique. Sometimes the three groups – atrophic, glandular hyperplastic and carcinomatous endometrium – can be distinguished even microscopically. Frequently the material is abundant enough to allow histological work. In other cases, the aspirated material is quite scarce and can be concentrated by washing and centrifugation.

COLETTE MARSAN, Paris, France:

The difficulties of obtaining endometrial smears are not sufficient enough, in our opinion, to justify the rejection of the method. We have systematically applied this method for the past two years to most of the women who were sent to our laboratory during or after the menopausal period; the only absolute contraindications being cervical infection or possible pregnancy.

Our technique is as follows:

The smears are taken with a metallic or plastic tube, bent so as to follow the curve of the cervical canal and fixed to a sealed syringe. Aspiration should not be done before the instrument has passed the isthmus, in order to obtain well-preserved specimens exclusively from the uterine cavity and not from the cervical canal or the cervix.

When possible, in the period prior to menstruation, aspiration should be taken immediately after the end of the menstrual period in order to avoid mucus in the cervical canal around the twelfth day and physiological desquamation of the altered endometrial cells immediately before menstruation.

Aspiration has always been painless, even in the few cases where grasping of the cervix has been necessary.

The fact that among 9 women with proven endometrial carcinoma we found more than one-half with negative cervical smears, whereas their endometrial smears were found positive, is significant enough evidence, in our opinion, to fully justify this procedure.

VIOLETTE M. NUOVO, Paris, France:

There is no doubt that endometrial aspiration greatly improves the accuracy of the detection of adenocarcinoma as Haour and Terzano have stated. In addition to vaginal and cervical smears, we try to obtain routine endometrial smears on all post-menopausal women. The aspirations have been performed without any notable incidents, but occasionally this procedure is impractical when stenosis of the canal exists.

Among the 139 cases of adenocarcinoma diagnosed cytologically, endometrial aspirations were obtained from 63 patients. The specimens were inadequate and the smears unclassified in only two cases. The smears of the remaining 61 cases were classed as either suspicious or positive for adenocarcinoma.

Moreover, among the 12 false-negative results ascertained cytologically, only three had endometrial smears, one of which was an inadequate specimen. Therefore, it is a logical conclusion that if endometrial smears had been taken on every patient, the percentage of the accuracy in the cytological detection of adenocarcinoma would have been increased. The resultant accuracy becomes 96 per cent, which is certainly significant. Thus, the importance of the endometrial smear is indicated.

We have never employed Haour's technique since our experience indicates that a very fine cannula attached to a syringe is an adequate method for obtaining endometrial smears. Blood and mucus do not impede our diagnosis. The three inadequate specimens in fact were cases in which a stenosis probably prevented further penetration into the endometrial canal.

Haour's technique is an interesting one. We have found, however, that when the aspiration was correctly performed, a sufficient amount of material was obtained. Furthermore, this procedure would seem impractical in a laboratory where the specimens are provided by several clinics and are consequently numerous.

GEORGE H. ROMBERG, White Plains, New York, U.S.A.:

I am in complete agreement with both Haour and Terzano that the advantages of the Endometrial Aspiration Technique far outweigh any disadvantages as commented on in both papers. Haour states that a hemorrhage may sometimes easily be induced by trauma of the aspiration technique. We have found that the routine use of an uterine sound introduced by the sterile technique helps gauge the position and depth of the endometrial cavity and thereby decreases appreciably the occurrence of hemorrhage in aspirating the endometrial cavity. The final comment I would like to make is in regard to Terzano's statement concerning the formal contraindication of the aspiration technique where suspicion of an early endometrial carcinoma is entertained, but cervical infection is present. The use of oral antibiotics has now made it possible in cases of this type to use the endometrial aspiration technique much more frequently than formerly.

HORST SMOLKA, Kiel, Germany:

Although we do not consider the endometrial aspiration technique a routine procedure, we have no doubts that this method is of great value in detecting endometrial cancer. Cervical or vaginal smears contain much more poorly preserved endometrial cells; quite often they contain none at all. On the other hand, the endometrial aspiration method is absolutely necessary for hormonal study of endometrial cells.

With regard to the technique of obtaining endometrial cells I would like to emphasize that the specimens are easily contaminated by endocervical cells when the cannula is being introduced and withdrawn. This is particularly true when withdrawing the cannula, due to less pressure in the tube. The contamination can be avoided by removing the syringe before withdrawal of the tube from the uterine cavity and placing the thumb over the tube opening.

The importance of the technique is emphasized by the diagnostic difficulties resulting if endometrial and endocervical cells are mixed. There is practically no possibility of contamination if an endometrial brush in a metal container is used.

CLOSING REMARKS

PIERRE HAOUR:

From the above discussions, it appears that endometrial aspiration is the more effective procedure for collecting endometrial cells and gives the best results in the diagnosis of adenocarcinoma of the uterus. The general opinion seems to consider endometrial aspiration, in the hand of the gynecologist, as a very simple procedure, which has in most cases, no serious inconveniences for the patient.

Various technical details proposed by the authors (time of application in the cycle, type of instrument, way of using it, procedures of concentration) will contribute to increase the accuracy of cancer detection.

The comparative study of endometrial and vaginal smears will probably be discussed more extensively in the next issues, but the results given by Violette Nuovo (96% correct results with the endometrial technique) and by Galbis (44% errors with the vaginal smears) are already significant.

Sometimes, however, endometrial aspiration is difficult to perform when a stenosis of the cervical canal is encountered, and also when one has to deal with a mass screening project. In those circumstances one has to resort to the cervico-vaginal smears. With the great number of errors present with this technique in adenocarcinoma detection, the methods proposed by Dellepiane, of which I have no experience, seem really excellent if cells can be obtained in great amount and well preserved.

As a final remark, I will say that in my own experience, if the endometrial smear is positive this does not exclude the histological control.

B. CORNELIS HOPMAN:

In reply to the discussion by Dellepiane: I agree that endometrial aspirations are justified if suspicion of an endometrial neoplasm exists or for endocrinologic reasons. The methods of Vecchietti and Barigozzi are not known to me but they certainly deserve attention. I agree that curettage may be indicated when aspiration fails to give a positive diagnosis and suspicion of cancer still exists.

In reply to the discussion by Galbis: I agree that endometrial smears cannot yet be used routinely.

In reply to the discussion by Colette Marsan: I agree that the advantages of the endometrial smear method surpass the disadvantages and that the endometrial smears will be more successful than cervical smears to find endometrial cancers.

In reply to the discussion by Violette Nuovo: I doubt whether gynecologic clinics in general perform endometrial smears as a routine. The method has certain risks, is time consuming, needs to be done by a physician and requires aseptic technique. Many physicians will prefer curettage to get more material even though endometrial aspiration smears have been able to diagnose cancer where curettage has failed.

In reply to the discussion by Smolka: The method of removing the syringe before withdrawal of the tube in order to avoid contamination with endocervical cells is certainly worth-while and important to remember.

GUILLERMO TERZANO:

It seems that there is general agreement regarding the advantages of endometrial aspiration. When dealing with problems related to early diagnosis of cancer, the end would justify the means.

Any method that could improve accuracy should be recommended and encouraged, even if it is an endometrial aspiration, brushing or washing, ergotamine tartrate, etc.

When contraindications are present (cervical infection, for instance), the gynecologist (or the general practitioner) will take the responsibility of the tests which he considers each patient needs (indication, previous treatment, etc.) in order to make them practicable and to help him reach a correct diagnosis.

Contraindications of intra-uterine aspirations are a question of common sense, as Iklé says. He is right and his statement concisely summarizes the whole discussion.

ADVANTAGES AND DISADVANTAGES OF INTRA-UTERINE BRUSH TECHNIQUE FOR ENDOMETRIAL CYTOLOGY

HANNS-WERNER BOSCHANN
West-Berlin, Germany

Cytological specimens obtained from the uterine cavity usually are mixed with cervical cells and mucus. This is caused by the fact that the cannula, which has a negative pressure during the entering, as well as, during the removal from the uterus, has to pass the endocervix. By this blending of corpuscular with cervical elements, the differential diagnosis between corpus and cervical carcinoma is aggravated. Even normal endocervical cells have been mistaken for endometrial cells in the secretory phase.

To avoid any disturbance by blending of the specimens, we have designed the following device: a sterilizable nylon brush, within a husk, is introduced into the uterine cavity. The husk (Diameter Hegar #3) is pulled back to a certain mark engraved on the handle of the instrument (in small uteri 4 cm., in large uteri about 8 cm.) so that the brush lies free in the uterine cavity. Then the brush is moved outward and the bristles scrape off some endometrial cells. At the same time, the brush enters the husk again and can now easily be removed without any contamination of the uterine specimen by endocervical constituents.

Advantages of the Method:

The cellular elements are better preserved than those taken by the aspiration technique. There are no more complaints than with a simple probe induction or with an induction of the aspiration

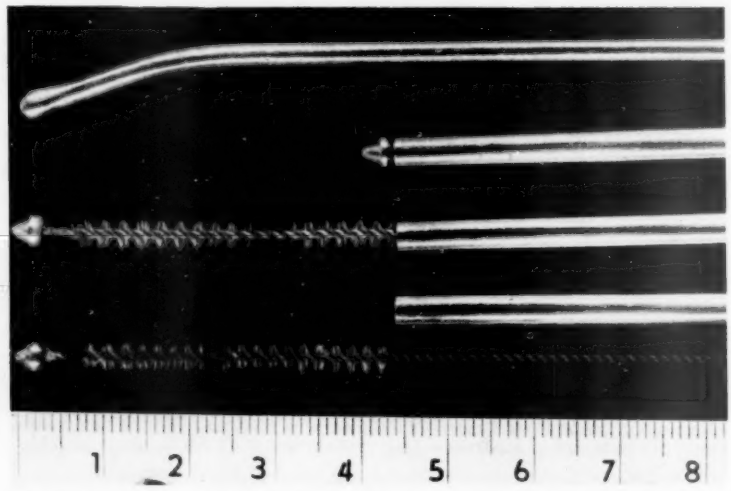

Fig. 1. From above: Aspiration cannula, closed endometrium brush, brush with husk pulled back, husk and nylon brush separate (Scale in cm and mm).

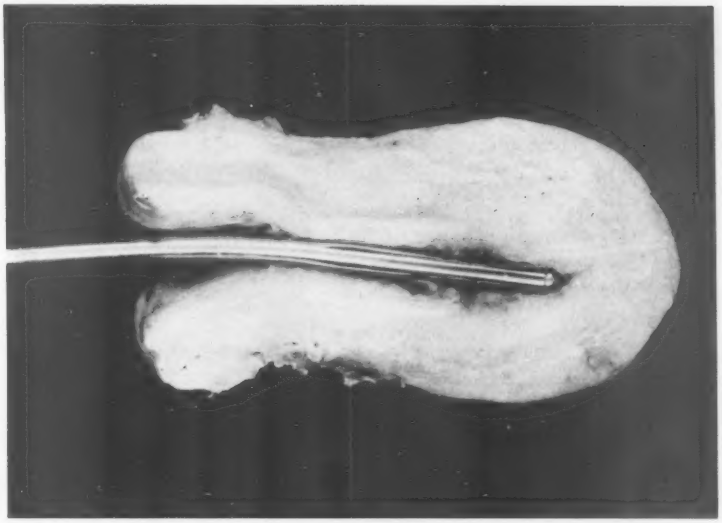

Fig. 2. The endometrial brush is introduced into the uterine cavity, lying within the husk, so that bristles do not come in touch with the endocervix.

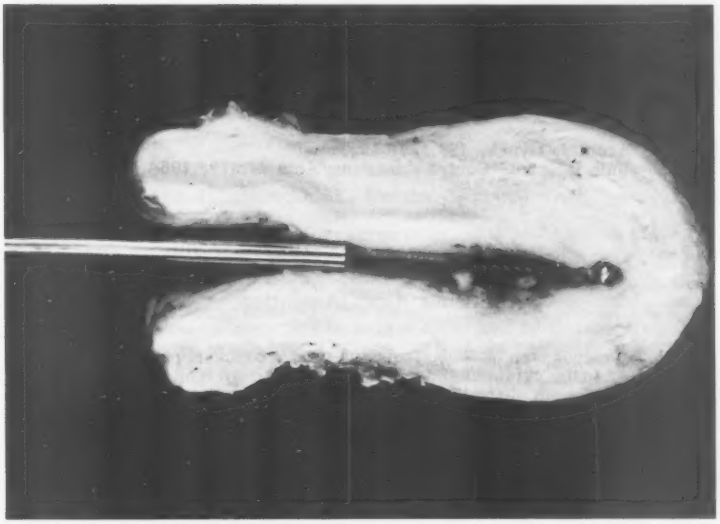

Fig. 3. The husk is pulled back (in small uteri 4 cm, in large uteri up to 8 cm - as shown by the mark on the handle of the instrument) and the bristles are lying freely in the uterine cavity.

cannula. Clamping of the cervix and dilation of the cervical canal are not needed, especially since the internal os is usually wide enough when bleeding warrants endometrial material for cytological study.

Disadvantages of the Method:

The brush has to be sterilized before every use (but this also applies to the aspiration cannula, as well as, to the polyethylene tube). For mass screening, there have to be a large number of instruments available.

The danger of perforating the uterine wall is the same as with the cannula but higher than with the polyethylene tube. Cannula and brush pass the cervical canal easily in multiparas and in patients with bleeding. With narrow cervical canals, a dilation occasionally may be necessary. The polyethylene tube can also be passed without additional measures in these cases.

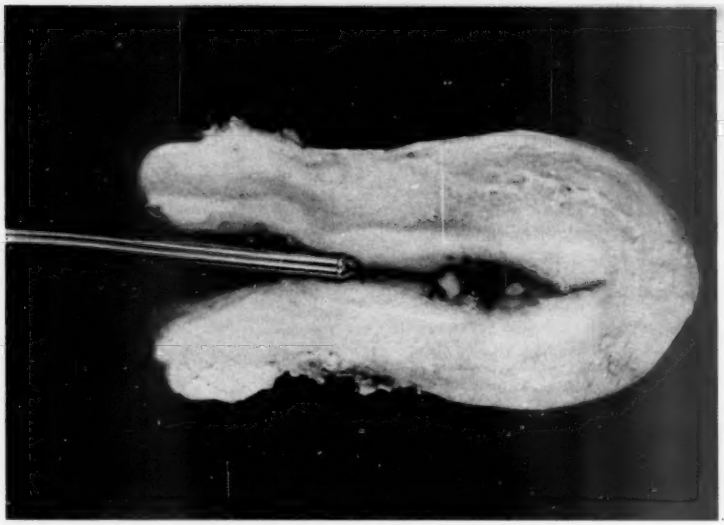

Fig. 4. The brush is pulled back while the husk stays in place and can now pass the cervical canal lying in the husk without cervical cells and mucus "contaminating" the bristles.

In our hospital and the clinics, as well as in the West-Berlin Cancer Detection Center, every examination unit keeps sterile endometrial brushes available. They are used in selective cases where very well-preserved cellular specimens are desired.

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JOSÉ MARIA E. MEZZADRA

Buenos Aires, Argentina

According to the monograph of Papanicolaou (5) and subsequent publications, the highest percentage of errors was noted in the cytologic diagnosis of the endometrium, and one of the reasons is (2) the fact that in nearly one fourth of the cases the exfoliated cells do not reach the vagina in a condition which permits a correct diagnosis.

In order to repair this inconvenience and obtain more abundant and clear cellular material, several technical devices have been employed, i.e., the rigid cannula described by Cary (3), the modification introduced by Hecht (4) and the polyethylene tube praised by Winer, et al (6). As a result of these experiments Ayre (1) designed the Rotating Endometrial Brush, with which we all are acquainted.

We consider its advantages to be the following: apparently it brings forth the exfoliation of a larger quantity of endometrial cells, even in lesions of an early stage, by accomplishing a real biopsy of the surface. The endometrial material does not become mixed with the material of other areas (endocervix, vagina, etc.). By having to insert the instrument, stenoses of the internal os are discovered, which are a frequent cause of false diagnostic negatives.

We consider its disadvantages to be the following: It cannot be carried out by technicians as a routine examination in mass screening, because it requires the intervention of a doctor. The endometrial carcinoma, which acquires its highest frequency after the menopause, is usually associated with cervical stenosis, making a dilatation, with the subsequent discomforts, hemorrhage, etc., inevitable. The surplus of cells through curettage are liable to mask the neoplastic cells arising from the tube, ovary or ascitic liquid. The cost, compared to a simple unbreakable cannula, is a further disadvantage.

Nevertheless, we may consider the Rotating Endometrial Brush a very useful and effective instrument, with which one can reach a high degree of cytodiagnostic accuracy in the diagnosis of benign conditions (polyps, hyperplasia, etc.) and especially of malignant lesions. Its systematic use will greatly improve the statistical results.

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HERBERT E. NIEBURGS
New York, New York, U.S.A.

The sampling of endometrial material by aspiration techniques often yields cells which have undergone post-exfoliative changes and therefore are difficult to evaluate. In addition, contamination by endocervical cells usually occurs.

With a brush technique the collection of abraded cells rather than exfoliatived cells is possible. If the brush is one in which the fibers may be retracted into a cannula, the contamination of the material by endocervical cells can be easily avoided.

The disadvantage of some brush instruments is the large diameter of the cannula which necessitates dilatation of the cervix prior to the introduction of the cannula. In order to eliminate the disadvantage of a large diameter cannula, we have constructed an instrument with cannulas of 2 mm and 3mm. This cannula is attached to a handle and permits up and down and rotating movements. In this manner thin nylon fiber loops may be extruded following the insertion of the cannula into the endometrial cavity. The specimen is obtained either by rotating or by scraping the endometrial wall in a similar manner in which a curette is used. With this procedure well preserved cells are usually obtained without contamination of endocervical cells. Although a larger number of cells are most often obtained by aspiration techniques than by our brush technique, it appears that the brush technique results in a greater percentage of satisfactory specimens. Cytometry of endometrial cells is more reliable when carried out in specimens obtained by brush techniques.

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

For clinical purposes, in the searchfor cancer cells, it is sufficient to use a simple cannula with a lateral opening at its tip or a polyethylene tube for the removal of cell material from the uterine cavity (as was shown in our experience).

For scientific investigations, however, one should use an instrument, which is certain to aspirate cells only from the uterine cavity. The technique described by Boschann appears to satisfy this condition. The use of the nylon brush is advantagous in that one gains better perserved cells and sometimes more material than by simple aspiration. The only disadvantage is the somewhat larger size of the instrument, which may require a dilatation of the cervical canal prior to its use.

Another method for preventing the aspiration of endocervical cells consists of the use of a double cannula which is covered with a rubber sheath. This latter is ruptured on reaching the internal os and the inner cannula then enters the uterine cavity. After aspiration the inner cannula can then be removed through the outer cannula without coming in contact with the cervical canal.

Recently we have used the instrument depicted below for the collection of cell material from the uterine cavity with good results. In place of the nylon brush there is a thin sponge. The instrument consists of a tube, equivalent in diameter to a Hegar's dilator #3. A second tube is passed through the first, its tip being fitted with a cap for closing the inner opening of the outer tube. Near the tip the inner tube is perforated in several places and these openings are covered with a thin sponge. With the instrument in the uterine cavity, aspiration by means of a syringe attached to the outer end of the inner tube will result in cell material collecting in the sponge while fluid is sucked into the inner tube. To prevent the simultaneous aspiration of endocervical cells, the closed instrument is inserted to the internal os and then only the inner tube enters the uterine cavity for aspiration. The instrument is closed before it is retracted.

This method almost always insures sufficient cell material without an excessive mixture of blood.

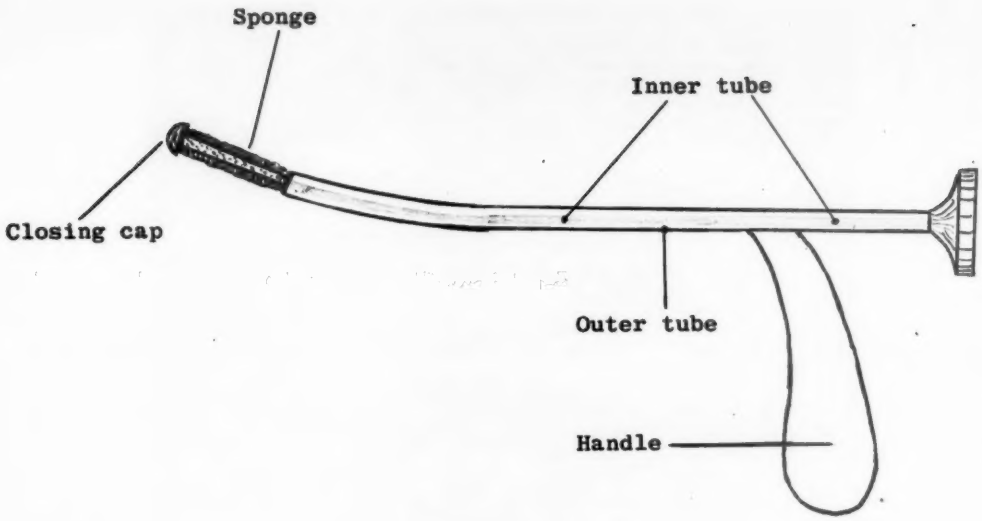

Fig. 1. (Bickenbach and Soost)

CLOSING REMARKS

HANNS-WERNER BOSCHANN:

I agree with Bickenbach and Soost that sometimes the cervical canal requires a mild dilatation if instruments equivalent in diameter to a Hegar's dilatator #3 are used as, e.g., the devices indicated in Bickenbach's and Soost's paper, as in my own. This difficulty, together with the necessity of sterile work, is an obstacle to endometrial cytology becoming a routine method.

JOSÉ MARIA E. MEZZADRA:

It is doubtless that for clinical and investigative purposes, the progress of cytologic studies of the endometrium will consist of removing the cell material from the uterine cavity. The type of instrument should be chosen individually for each patient, since even the most slender instrument may require a dilatation of the cervix, whereas on the other hand a dilated cervix may permit the insertion of any instrument.

The instrument described by Bickenbach and Soost appears to be most interesting, but it should not be forgotten that many small lesions in a lateral cornu can be easily reached by means of an endometrial brush, collecting a greater quantity of material and thus avoiding errors. It is well-known that many small carcinomas have not been recognized, despite uterine curettage, especially when a careful exploration has not been carried out and when a previous hysterography to localize the site of the lesion was not available.

We believe, therefore, that it should be left to the sagacity of the gynecologist to decide which instrument to use in each individual case.

ADVANTAGES AND DISADVANTAGES OF THE CERVICAL PESSARY AS A COLLECTION TECHNIQUE FOR ENDOMETRIAL CYTOLOGY

FRIEDRICH BAJARDI
Graz, Austria

In order to improve the accuracy of cytology in the diagnosis of intra-uterine carcinoma, the technique of cervical pessary was employed in the author's investigations, following a suggestion by Navratil (1). In cases of suspected corporal carcinoma a cervical pessary was used to collect secretions during a period of 6-8 hours which were then examined cytologically. Although the number of cases is still insufficient to prove the efficiency of this method for statistical evaluation, certain advantages and disadvantages are already clearly evident:

Disadvantages of technique:

1. Covering the cervix by means of a pessary produces an accumulation of secretion, with a subsequent local inflammatory reaction in most cases.
2. Abundant leukocytes and microorganisms due to such inflammatory reactions may render the examination of smears difficult.
3. Accumulation of secretions may further cause lysis of normal ectocervical epithelium. Exfoliated vaginal squamous cells will be prominent in such cases.
4. Endometrial cells collected in the pessary are subject to disturbing influences due to accumulation and inflammation. Degenerative changes of cells may be very pronounced, thus making the cytological evaluation difficult.

Advantages of technique:

1. The cervical pessary method is without danger and may be repeated as often as required.
2. This method may also be employed in cases where intra-uterine smears or curettages may be difficult to obtain because of stenosis in the cervical canal.
3. The quantity of secretions collected in the cervical pessary (usually 1/2 to 1 cc) will provide sufficient material for numerous smears leading possibly to an improved accuracy of technique.
4. Endometrial cells are usually present in larger numbers in individual smears as compared with smears obtained by the aspiration technique from either the posterior fornix or the cervical canal. This applies especially to exfoliated tumor cells.

In summary, the pessary technique is suggested as an improvement in the early cytological diagnosis of cancer, without having the disadvantages of intra-uterine smears. This technique seems to show an increased diagnostic accuracy as compared with the extra-uterine methods of preparation of cytological material.

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DISCUSSION

HANNAH PETERS, Bombay, India:

I would like to ask Dr. Bajardi a question:

He states that endometrial cells are usually present in larger numbers in smears prepared from the cap. Is this true only in cases in which there is some endometrial pathology, or does he find endometrial cells also in material collected in the cap from normal women? In our experience of more than 200 cap smears (these were not done for cancer diagnosis but in the course of testing the harmlessness of contraceptives) on healthy women, no endometrial cells were found in any of the smears (1). Although many leukocytes and histiocytes were seen in these smears, we found that the smears were usually readable; though the cap was left in place for 24 hours in our cases, cytolysis or autolysis, in most cases, did not make the collected endocervical cells difficult to interpret.

I agree that the method of preparing smears from a cervical cap is useful in all cases in which more material is desired than is available by the direct preparation of the smear.

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CLOSING REMARKS

FRIEDRICH BAJARDI:

As mentioned previously, cervical pessary collection method always has been applied in those cases, where corporal carcinoma was suspected. Thus, in all cases symptoms have been present, e.g., usually bleeding anomalies; especially post-menopausal bleeding. These same symptoms have been proven to occur in mucosal hyperplasias, endometrial polyps and submucosal myomas. The endometrium, therefore, has been altered in all these cases.

However, this method has not been tested on healthy women.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

THE DIAGNOSTIC ACCURACY OF VAGINAL SMEARS FOR DETECTION OF ENDOMETRIAL CARCINOMA

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

It has been generally thought that though vaginal smears are accurate in the diagnosis of cervical cancer, they leave much to be desired in the diagnosis of endometrial cancer. There have been many publications indicating the diagnostic accuracy to be somewhere between 50 and 80%. It has never approached the accuracy of the diagnosis of cervical cancer. In 1953 I published the diagnostic accuracy of 218 endometrial carcinomas. There was a false negative figure of 23% (1).

However, in the years since 1953, I have had the distinct impression that it was far better than the figure above would indicate. For the purposes of this discussion, I have reviewed the accuracy in all primary corpus carcinomas seen in the year and a half since I have been at the Roswell Park Memorial Institute. During that period of time there have been forty-nine primary cases of carcinoma of the corpus. Of these, four have been called negative or a diagnostic false negative error of eight per cent. One case was called doubtful and was not repeated before a dilatation and curettage had been performed. All the remaining forty-four were reported as positive.

Two of these cases are particularly interesting from the cytologic viewpoint. One patient had had five dilatations and curettage for post menopausal bleeding. In every instance the histology report was "Endometrial Hyperplasia." The cytology showed cells typical of adenocarcinoma on three separate occasions. A total hysterectomy was performed and a small endometrial carcinoma was found.

In the second case the cytology was the only indication that anything was abnormal in the genital tract. The patient was referred to the Institute for question of post-operative radiation after a radical mastectomy. A routine vaginal smear was positive and contained adenocarcinoma cells. On strict questioning the patient admitted to slight spotting several weeks before, but this fact only came to light after persistent questioning and after the diagnosis of the smear was known. On physical examination the vagina appeared atrophic and the uterus small and movable. The examining physician thought the diagnosis of corpus carcinoma extremely unlikely and interpreted the cells appearing in the vaginal smear as perhaps desquamating from a co-existent urethral caruncle. Routine dilatation and curettage revealed an adenocarcinoma of the endometrium.

A third case not included in the forty-nine, because it is a recurrence, is of interest. A vaginal smear was reported as positive and consistent with adenocarcinoma on a patient treated with radium for a carcinoma of the endometrium fifteen years previously. The patient reported that she had had a slight vaginal spotting recently. At ether examination the cervical os could not be identified and no source of the bleeding could be found except for the atrophic vaginal mucosa. A second vaginal smear contained adenocarcinoma cells. A second ether examination again proved fruitless in either finding the source of the tumor cells or the slight amount of bleeding. A hysterectomy was performed and a small recurrent adenocarcinoma of the endometrium was found.

This last case is of particular interest. Though on two examinations under anesthesia it was impossible to identify the cervical os, yet large numbers of tumor cells were desquamating into the endometrial cavity and finding their way through to the vagina. We sometimes forget that because an orifice appears to be sealed off grossly, it is no assurance that it is sealed off microscopically.

That these figures are better than previously reported is due to one major factor, in my opinion. In our laboratory we do not expect to see groups and clusters of tumor cells in adenocarcinoma but screen the slides carefully for single tumor cells which we call "single differentiated adenocarcinoma cells."

These cells came to my attention because of a particularly frustrating error. Vaginal smears had been taken every six months on a post-menopausal patient and in every instance they had been considered negative. Five years after the first smear had been taken, a smear was taken which to our chagrin was filled with adenocarcinoma cells. Careful review of the previous slides showed that the majority of them contained small, single cancer cells which had been interpreted as histiocytes. Since

this particular case, screening technicians have been especially instructed to look for these cells. I feel quite certain this has accounted for our increase in accuracy.

The single adenocarcinoma cell is not large, being only slightly larger than a histiocyte. It is often mistaken for the well preserved histiocyte since its nucleus is eccentric and sits on the very edge of the cytoplasm. No cytoplasmic border can be identified surrounding the nucleus. The cytoplasm is apt to appear thin and to stain somewhat irregularly in contrast to the foamy, filmy cytoplasm of the histiocyte (Fig. 1). It is the nuclear pattern, of course, which identifies the cell as malignant. The chromatin is irregularly arranged. It is in heavy clumps and strands and the background is not homogenous (Fig. 2).

I am convinced that if particular attention is paid to this single differentiated cell of adenocarcinoma, the diagnostic accuracy of endometrial carcinoma by examination of the vaginal smear can be considerably increased.

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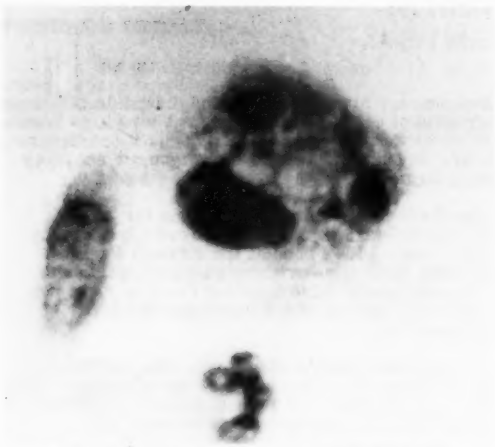

Fig. 1. A phagocytic histiocyte. The nucleus is eccentric in position and occupies an eccentric position on the edge of the foamy cytoplasm. The chromatin of the nucleus is regular in pattern. X1080

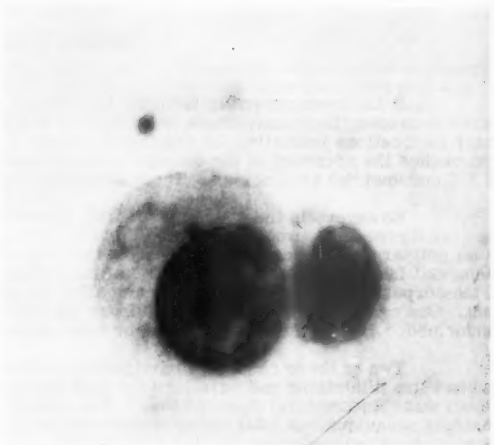

Fig. 2. A single differentiated adenocarcinoma cell. The nucleus is eccentric and is at the edge of the cytoplasm. The irregularity of the nucleus identifies the cell as malignant. The smaller cell is a small histiocyte with a bean-shaped nucleus. X1080

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

The diagnostic accuracy of vaginal smears for the diagnosis of carcinoma of the endometrium is admittedly below the accuracy for the diagnosis of carcinoma of the cervix, and in various published statistics stands between 70 and 75 per cent (1). In a carefully controlled study Jordan and coworkers (2) state that one may now expect an accuracy of 76% using vaginocervical smears and an accuracy of 84% using endometrial smears. The two tests combined with endometrial section biopsy give 100% accurate diagnosis.

The purpose of this paper is to evaluate the accuracy of vaginal smears in the diagnosis of endometrial carcinoma as it was observed in our cytological laboratory. In order to do so, we analyzed all cases of endometrial carcinoma which had come under our observations for the past 5 years and in which cytological studies were performed before biopsy. Thirty-five cases came from patients participating in the large cancer survey conducted by the Departments of Pathology and Gynecology of Ohio State University and the National Cancer Institute on the female population of Franklin County, while 27 cases came from patients referred to this laboratory by private physicians or hospitals. In the cancer survey group only vaginal aspirations were available in most instances while all the referred patients had vaginal aspirations as well as cervical scrapings, and in 15 cases endometrial smears were also available.

In studying these cases, we followed the suggestion of Jordan and coworkers, tabulating first our original diagnosis and listing below the diagnosis corrected after thorough review of the slides by the

author after it had become known that the patient had a positive biopsy. By this method the true false-negative slides caused by inadequate collection of the material can be separated from the false-negative caused by human error. The number of true false-positive positives then will give the true diagnostic value of the method, while the difference between the reviewed and unreviewed data reflects upon the efficiency of the staff.

Material

A total number of 62 cases was reviewed. The age of the patients varied between 45 and 81, the majority belonging to the white race. The marital status was of no significance. The 62 women had delivered 133 children with a birth rate of 2.14 children per woman, and the entire group listed 20 abortions or miscarriages. Twelve patients still menstruated at the time the diagnosis was made; 9 of them complained of menstrual irregularities. The oldest of these patients was 57 years. The other 50 cases were from 1 to 31 years in the menopause. The clinical diagnosis of endometrial cancer was suspected in 22 cases, or 33 per cent, the suspicion based upon the history of postmenopausal spotting during the preceeding year. Careful interrogation elicited an occasional spotting in 39, or 63 per cent, of our patients. Sixteen patients showed a severe purulent discharge. The results of our analysis are listed in Table 1.

In our laboratory, the accuracy of the vaginal smears for the diagnosis of endometrial carcinoma stands at 74 per cent. After all slides had been reviewed a diagnostic accuracy of 81 per cent was obtained. Cervical scrapings have proven far less reliable and even careful review of the smears did not increase the accuracy appreciably. The few endometrial smears which were available for analysis showed them decidedly superior to the other methods of collection of material in diagnostic value.

Attempts at differentiation between carcinoma in situ and invasive carcinoma of the endometrium have been unsuccessful. Attempts to recognize the histological type of endometrial carcinoma by studying the malignant cells present in the cytological preparation have been only partially successful, we were able to recognize 6 of the 9 cases of adenocanthoma by the presence of malignant squamous cells. In the usual adenocarcinoma both small, or undifferentiated, and large, or differentiated, cells (3) were found, and the quantitative estimation of these types proved impracticable.

False-positive smears were obtained in our laboratory in only 6 cases which subsequently proved negative on curettage and cytological reexamination. This is in contrast to cytological examination of the cervix, where one encounters the difficult problems of cellular dysplasia.

In summary then, we wish to state that in our laboratory the diagnostic accuracy of vaginal smears for the diagnosis of endometrial adenocarcinoma was found to be 74 per cent with the possible increase up to 81 per cent after review of the cases by an experienced cytopathologist. This is less than the accuracy obtainable by the study of endometrial smears but considerably higher than that obtained by a study of cervical scrapings.

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TABLE I

COMPARISON OF DIFFERENT METHODS OF CYTOLOGICAL EXAMINATION AS TO THE ACCURACY IN THE DIAGNOSIS OF 62 CASES OF ENDOMETRIAL CARCINOMA

Type of Cytological Preparation	Positive		Suspicious		Positive and Suspicious		Negative	
	No.	%	No.	%	No.	%	No.	%
Vaginal aspirations	35	57	11	17	46	74	16	26
Vaginal aspirations after review	43	69	7	12	50	81	12	19
Cervical scrapings	21	48	6	13	27	61	17	39
Cervical scrapings after review	21	48	8	17	29	65	15	35
Endometrial smears	10	67	2	13	12	80	3	20
Endometrial smears after review	10	67	3	19	13	86	2	14

ERICA WACHTEL
London, England, U.K.

In the past five years, 49 new cases of endometrial cancer were admitted to the Gynaecological Department of the Hammersmith Hospital (London). Of these 49 patients, 46 had "positive" vaginal smear findings with a cytological diagnosis of adenocarcinoma on first examination and three were missed cytologically. This corresponds to a diagnostic accuracy of 93.9% or a rate of error of 6.1%. Two of these lesions were clinically unsuspected. During the same period we had, however, 8 "false positive," i.e., histologically unconfirmed reports of adenocarcinoma. Four of these came from atypical hyperplasia, one from a polypoidal endometrium, one from a case of metropathia and two had histological diagnoses of severe endometritis.

DISCUSSION

MARCEL GAUDEFROY, Lille, Nord, France:

The papers of Ruth Graham, Erica Wachtel and von Haam are very interesting for different reasons. Ruth Graham shows the considerable significance of the described type of single differentiated adenocarcinoma cells. It is very useful to prove that it is possible to increase the diagnostic accuracy with this method. But not all cytologists have the "maestria" of Ruth Graham. If the diagnosis is determined by the observation of a very few single cells, must we not fear increasing the percentage of false positives?

Erica Wachtel is to be congratulated for her high percentage of diagnostic accuracy in detection of endometrial carcinoma by vaginal smears. In my laboratory, the diagnostic accuracy is not more than 80%, but we have 0% false positive. The 20% leavings are diagnosed by endometrial smears, endometrial biopsy, or curettage.

Von Haam corroborates the opinion that it is easier to find something if the inquirer already knows what he is looking for is present. This is the reason that diagnostic accuracy is better when the cytologist, if he is a gynecologist, has personally examined the patient clinically before screening the smears.

JOSÉ MARIA MEZZADRA and GUILLERMO TERZANO, Buenos Aires, Argentina:

When we study smears in order to diagnose early endometrial cancer, we keep in mind that we are exposed to the risk of a false negative report. For this reason, we usually take several smears from each patient and perform an endometrial aspiration whenever it is possible.

In our material the smears of 108 women with a proven carcinoma of the endometrium have been reported as follows:

Class	I	II	III	IV	V
No. of cases	4	10	15	49	30

Our accuracy has been: with vaginal smears - 52.8%, with both vaginal and endometrial smears - 73.1%.

The diagnostic accuracy of the method was 79.6% (or might be 87.9% if the Class III could be included), because the smears were reviewed after knowing the pathological report and cancer cells were found in one of the negatives and six of the Class III cases. In the original negative case the cancer cells were missed; in the six Class III the cells were not classified as positive because of over emphasis upon keeping within safe bounds. None of the Class III remains suspicious.

LUIS MONTALVO-RUIZ, Madrid, Spain:

The papers of Ruth Graham, von Haam, and Erica Wachtel are very interesting because they give very satisfactory figures of positive reports. In the statistics we have compiled from a few cases, we were able to attain only 50% accuracy in the use of vaginal smears for detection of endometrial carcinoma. Since we do not believe that vaginal smears give us sufficient accuracy for diagnosis of endometrial carcinoma, we do not use them for this diagnosis as frequently as we do for cervical carcinoma.

On the other hand, we believe that endometrial smears have a great value and accuracy. With Wied, we believe that as the smear is taken closer to the uterine cavity, the diagnostic accuracy of endometrial carcinoma increases. Thus, Wied tested 6-29% of his cases by vaginal smears, 55% by cervical smears and 84% by uterine aspiration smears.

However, the figures of Ruth Graham, von Haam and Erica Wachtel encourage us to undertake this study again and to make our results known later.

VIOLETTE M. NUOVO, Paris, France:

The results of our investigation concerning the detection of endometrial carcinoma by means of vaginal smears are directly comparable to von Haam's studies. Of the 32,333 patients who had gynecological smears, 151 had adenocarcinomas of the fundus. 139 cases out of the 151 had suspicious or positive smears.

NUMBER OF CASES	CERVICAL SMEARS	VAGINAL SMEARS	ENDOMETRIAL SMEARS
32	+	+	+
1	+	+	inadequate
13	-	+	+
1	-	+	inadequate
2	+	-	+
8	-	-	+
19	-	+	0
7	+	-	0
42	+	+	0
8	0	+	0
6	0	0	+

Observing this chart, one notes that in 116 cases, the vaginal smears were sufficient to diagnose an endometrial carcinoma. Thus, the accuracy of the vaginal smears in our laboratory has been 76%. If both cervical and especially endometrial smears are obtained along with the vaginal smears, the accuracy is increased to 92%.

The conclusion is therefore self-evident. To obtain the most accurate results, three specimens (vaginal, cervical and endometrial) from each patient and particularly from postmenopausal women seem necessary for the detection of endometrial carcinoma.

CLOSING REMARKS

RUTH M. GRAHAM:

One of the important points raised in this discussion is whether or not the false positive reports are increased if the diagnoses are made on a "very few single cells." I would like to make it clear that because we emphasize the single differentiated cell of adenocarcinoma this in no way implies that they are few in number. Actually in the majority of cases of corpus cancer, there are many such cells.

As regards the false positive reports, there were two during the period of time that 46 of 50 cases of corpus cancer were correctly called positive. This is a diagnostic error of 4% which I do not regard as prohibitive. One patient had an atypical hyperplasia of the endometrium, and the second, endometrial polyps.

Many of the discussors state that the accuracy is increased if all types of smears are considered, and their figures seem to justify this statement. However, since in my own material, one vaginal smear equals the diagnostic accuracy of three different types of smears, it would appear possible to obtain accurate figures on one smear. I feel this is an important point. Our goal is to make the method available to the greatest number of patients. It is probably better to do one smear on three thousand patients than three smears on one thousand patients. We should make an effort to increase the diagnostic accuracy of one smear. Careful attention to the cytological criteria of malignancy will increase the accuracy, but probably of equal importance is that the smear be taken and fixed properly. I suspect that not enough emphasis is placed on the simple technical factors involved in obtaining a vaginal smear.

EMMERICH von HAAM:

The figures of Mezzadra and Terzano as well as those of Violette Nuovo strongly speak for the advantages of endometrial aspirations for the diagnosis of endometrial carcinoma. I believe that this procedure should be done in any woman over 50 years of age, but especially in any woman over 50 who has maintained a high estrogenic type of smear or who is spotting. Unfortunately the technique of endometrial aspiration requires more time and professional skill and is therefore still little used.

ERICA WACHTEL:

While I fully agree with Violette Nuovo, Terzano and Mezzadra that the diagnostic accuracy of endometrial cancer, by means of cytology, can be enhanced by including endometrial aspiration smears to the screening material, I would like to stress some points in relation to posterior fornix smears. Results in detecting adenocarcinoma cells will be improved if the screener is on the lookout for them and bears in mind not only the morphological characteristics of the carcinoma cells, but also the general smear appearances of carcinoma patients.

Such warning signals include a high Karyopyknotic Index and other manifestations of a high estrogen level in postmenopausal patients and the presence of red blood cells. Admittedly these features in the smears do not necessarily mean malignancy, since hyperplasia of the endometrium and endometrial polyp, in the absence of definite evidence of malignancy, may produce similar smear patterns, but when found, they should be noted with concern and repeat smears, and if possible an endometrial aspiration should be taken. I would also like to issue a note of warning not to be too rash in the classification of undetermined groups of cells as histiocytes. A misinterpretation of histiocytes and adenocarcinoma cells can easily recur. Violette Nuovo's suggestion to take three specimens (vaginal, cervical and endometrial) from each patient is a good one, but it imposes a heavy extra load on the daily routine of busy diagnostic laboratories. I, therefore, propose relying on vaginal smears in the first instance but resorting to additional procedures such as endometrial aspirations where the post-fornix smears are inconclusive.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

THE DIAGNOSTIC ACCURACY OF CERVICAL SMEARS FOR DETECTION OF ENDOMETRIAL CARCINOMA

JULIETA CALDERON de LAGUNA
Mexico, D.F., Mexico

The data presented here form part of a study by members of the staff of our laboratory. This data is to be published elsewhere in full.

In the National Institute of Cancer of Mexico City, we have always taken samples from two sources: a smear from the vaginal pool and another smear directly from the cervix, obtained through the vaginal speculum. Papanicolaou's pipette is used for both. Some time ago, in order to compare the diagnostic accuracy of each type of smear in genital cancer, we studied 1250 positive smears chosen at random (most of them squamous carcinomas of the cervix) with the following results:

Malignant cells in both smears – 1016 cases or 81.2%

Positive diagnosis established in smear No. 1 exclusively – 78 cases or 6.2%

(Most of these smears belonged to bloody or highly necrosed tumors. We included those cases in which the cervical smear was doubtful positive and the vaginal smear was definitely positive, as well as those cases in which malignant cells could only be found in the vaginal smear.)

Hysterectomized patients, irradiated patients, virgins and all other cases, in which, for various reasons, only one smear was taken were not included.

In a recent review of our material, we were able to find 72 endometrial adenocarcinomas with histological confirmation by means of a biopsy or surgical specimen. Cytological studies were always carried out as stated above; usually the histological study was made afterwards. In this group, diagnosis invariably was made from the smear obtained by cervical aspiration, except for three cases in which the speculum was not used due to the fact that the patient was a virgin.

Cytological diagnoses were as follows:

	Number of cases
Positive IV Carcinoma	1
Positive IV or V Adenocarcinoma	46
Positive V Adenocarcinoma of the Endometrium	10
Positive IV Adenocarcinoma of the Endocervix	1
Positive IV Squamous Carcinoma	1
Doubtful III-IV Adenocarcinoma	7
Negative III Glandular or Endometrial Hyperplasia	5
Negative II Endometritis. Abundant Histiocytes	1

Evidently a correct and specific diagnosis was made in 56 out of the 72 cases, that is, in 77 per cent of the cases. Smears diagnosed as doubtful, the case of non-classified carcinoma, and the case of adenocarcinoma wrongly attributed to the endocervix, belonged, of course, to cases of cancer, but the diagnosis was not specific. There was only one case of a wrongly classified smear, that one being reported as squamous.

In this series, we reported 6 out of 72 (8.3%) as false negative. In the whole group of carcinomas located in the endometrium and also adenocarcinomas of the endocervix, the cytological diagnosis error goes down to 6%. This group is made up as follows:

Location of tumor	Number of cases	False Negative
Endometrium	72	6
Endocervix	22	0
Ovary (endometrial metastases)	6	0
Rectum (uterine invasion)	1	0
Endometrial and Endocervical adenoacanthomas	10	0
Endometrial Chorioepitheliomas	3	1
	114	7

The cases correctly diagnosed as negative were not included in calculating this percentage of false negative cases. When we made a negative diagnosis, this means an over-all negativity, including the endometrium. Taking this fact into consideration, our final total of false negative is 3.5% (1).

Nevertheless, we have considered as a specific group, those cases in which clinically there was some question about the body of the uterus being involved, i.e., benign tumors, inflammatory conditions, hyperplasias, etc. Furthermore, in these cases we were able to observe endometrial cells, but with no malignant characteristics. Ordinarily we only find columnar cells from the endometrium in smears taken with the cervical aspiration technique during the menstrual phase or in pathological conditions. This group contained 111 cases, including the 6 false negatives mentioned above. The diagnostic error (false negative) in this case is 5.3%. Of these 6 false negative cases, two were cases in which the endometrial biopsy was also negative and the clinical diagnosis was confirmed by the surgical specimen.

In this series, we reported 16 doubtful adenocarcinomas in which biopsies were taken. Histological diagnoses revealed 7 positive cases, 8 negative cases and one doubtful case.

In 8 of the 72 cases of endometrial adenocarcinoma, clinicians had not suspected any malignant condition and diagnoses were primarily made by means of cytology.

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany

In 96 cases of cytologically examined endometrial carcinomas of our material, the following smears were positive:

a. Smears from the posterior fornix and ectocervix..... 31 (32%)
b. Smears from the endocervix 59 (61%)
c. Smears from endometrial aspirations..... 83 (86%)

Wied has published similar results about the first 32 cases of this material.

If the diagnostic accuracy of different authors varies between 50 and 100% in endometrial carcinoma, this may be due to their different smear collection techniques. Smears from the posterior fornix and from the ecto- and endocervix show a rate of error of 40-50%, as most authors agree (1, 2, 3, 4, 5, 6, 7, 8). They have, on the other hand, led to the detection of a number of cases not yet clinically manifest (9, 10).

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LUIS MONTALVO-RUIZ, Madrid, Spain:

Detection of endometrial carcinoma by cervical smears is very difficult; however, it is more exact. We can obtain 60% accuracy with them.

We have observed that results are influenced by the way in which the smear is taken. I believe that taking endocervical smears with a swab of wet saline solution is better than using Papanicolaou's pipette. We use the latter only when taking vaginal smears.

An interesting point of the paper, and of great value for us, is the cytological diagnoses of endometrial carcinoma in 8 cases in which there were no clinical suspicions of malignancy. I have two similar cases in which the diagnoses of malignancy were done first by cytology and later proven by histology.

I congratulate Julieta de Laguna on the large number of cases that she has been able to manage.

YOSEUP S. SONG, Providence, Rhode Island, U.S.A.:

During its first year of operation, the mass screening survey of the State of Rhode Island completed 25,000 cases in which 19 cases of adenocarcinoma of the fundus were discovered. Eight of the 19 cases had been clinically suspected, but the remaining 11 cases were found among asymptomatic women. Both the vaginal aspiration and cervical scraping smear were made routinely from asymptomatic women and then examined by our laboratory technicians. A comparison study has been attempted, and the following are the data which we obtained:

CYTOLOGY READINGS

(Adenocarcinoma of Fundus - 19 cases)

Cytology Classification	Cerv. Scrap. - Pos. Vag. Aspir. - Neg.	Cerv. Scrap. - Pos. Vag. Aspir. - Pos.	Cerv. Scrap. - Neg. Vag. Aspir. - Pos.
Number of Cases	4	10	5

The above data appear insufficient in establishing a diagnostic accuracy of cervical scraping smears in the detection of adenocarcinoma of the fundus. However, it is quite obvious that the value of cervical scraping smears in detecting fundal carcinoma should not be underestimated, since four cases of adenocarcinoma would have been overlooked if the cervical scraping smear would not have been available.

GUILLERMO TERZANO, Buenos Aires, Argentina:

When we repeated the investigation of Wied (as mentioned in "Endometrial Aspiration Technique") we diagnosed by cervical aspiration only 8 of 12 carcinomas of the endometrium.

We did not keep a record on cervical aspirations as complete as de Laguna, but we made correct diagnoses in those cases in which, because of bleeding, it was possible to obtain cells from the uterine cavity. It is the same with cases involving endometrial hyperplasia and endometrial polyps.

LUBA A. TURNBULL, Montreal, Canada:

The accuracy of diagnosis of endometrial carcinoma by cervical smear varies with different authors from 45-85%. This variation depends highly upon the experienced cytologist himself and also on how well the technician prepares the smear.

Endometrial cytology needs exceptional concentration in the study of the cells.

If the cytologist is tired after viewing many smears, it is better not to make a quick decision.

In our laboratory we use a wooden spatula for preparing the cervical smear and a pipette for aspiration of the external os. If the cells are passing easily through the endocervix into the vagina, the two smears should show endometrial cells.

During the past 6 years of our work, our accuracy has not been constant and from year to year has varied from 12-71%.

NO CLOSING REMARKS

ARE DEGENERATIVE CELL CHANGES IN ENDOMETRIAL CELLS DUE TO INADEQUATE PREPARATION TECHNIQUE OF SMEARS?

J. PAUL PUNDEL
Luxembourg, Luxembourg

While the squamous epithelium of the vagina and the uterine cervix presents a continuous desquamation of the outer cell layers, the endometrial epithelium is characterized by a different desquamation tendency which exists in the normal woman only in the menstrual phase. From this biological fact, one can conclude that all endometrial cells seen in a vaginal or cervical smear taken outside of the normal menstrual phase can be considered as evidence of a pathological process in the endometrium: inflammation, hyperplasia, or even cancer. In these cases, the desquamation of the endometrium can be continuous, even in the absence of a clinically visible bleeding. This is a rather useful phenomenon for the early cytological diagnosis of endometrial cancer.

Unfortunately, this advantage of continuous desquamation is greatly counterbalanced by degenerative phenomena which appear in the desquamated endometrial cells, thus making the differential diagnosis between benign and cancerous cells very difficult, especially if the cytological diagnosis has to be based only upon vaginal smears.

Are these degenerative cell changes frequently observed in the endometrial cell due to inadequate preparation techniques of the smear or due to biological phenomena? This important problem for the cytological diagnosis of endometrial pathology can be resolved by:

(a) the study of the normal pathological conditions of the desquamation of endometrial cells and the cell behavior between the moment they are shed and the moment they are collected for the cytological examination.

(b) the study of endometrial cells collected at different levels of the utero-cervico-vaginal tract and prepared by different techniques.

A. The normal and pathological conditions of the desquamation of the endometrial cells.

Even in the typical normal conditions of endometrial desquamation during the menstrual phase, the shedding of the endometrium is preceded by autolytic phenomena involving mainly the cytoplasm and later the cell nucleus. This explains why, during the normal menstrual phase, the major part of the endometrium is eliminated not as tissue debris but as a more liquid form resulting from a more or less complete autolysis due to ischemia and enzymatic processes. The cells thus shed during the normal menstrual phase present more or less degenerative changes at the moment of their desquamation, as observed by direct intra-uterine collection of these cells. If we consider now the smears taken at lower levels, at the external orifice of the cervix or even in the vagina, the degenerative phenomena of the endometrial cells are more important. This degeneration is due to the relatively long distance the cells have to pass before they arrive at the outside of the uterus or are collected for the smear. During this time, the enzymatic processes of autolysis continue, and when the cells arrive in the vagina and are not collected immediately, they are, furthermore, exposed to other destructive factors, such as changes in the pH, bacterial destruction due to lack of oxygen. The degeneration, necrosis or autolysis of the endometrial cells observed in the usual cytological preparations is favored by the biological structure of these cells, which are not as well protected against exogenous factors as the squamous vaginal cells. They are glandular cells which have a rather thin cell "membrane" and their cytoplasm contains substances such as mucus and glycogen, which are easily autolyzed by intra-uterine and even

vaginal enzymes.

The desquamative tendency of the endometrial cells and other factors which can produce degenerative changes in these cells (even locally before the exfoliation) are accentuated under pathological conditions, e.g., endometritis, hyperplasia and cancer. In these cases the autolysis observed in the normal menstrual phase is not marked because the biological conditions exist at their maximum only in the normal secretory premenstrual endometrium. Other factors appear in these cases, e.g., bacterial or inflammatory destruction and necrosis by ischemia of the outer cell layers. (These layers cannot receive a sufficient blood supply due either to the marked proliferation, as seen so frequently in cancer cases, or to thrombosis of the endometrial vessels in cases of hyperplasia or cancer.) Also the cells of hyperplastic and cancerous origin can contain marked amounts of mucus or glycogen, which renders these cells more sensitive to destructive enzymatic factors, inside as well as outside of the uterine cavity.

B. Influence of the cytological technique upon the degenerative changes of endometrial cells:

In order to discover the possible destructive factors of the cytological technique, I have studied, with Herovici and Lichtfus, many cases of adenocarcinoma and endometrial hyperplasia by means of several techniques. We compared the smear pictures (a) with those of direct preparation from the endometrium and (b) with histological slides. Our results are the following:

(1) We could not find any apparent influence of the preparation technique of the smears (vaginal, ecto- and endocervical and intra-uterine) when the technique was correctly done as described for the Papanicolaou or Hematoxylin-Shorr method. Only inadequate preparation of the slides by carelessly rubbing the cells on the glass or by neglecting the immediate fixation accentuated the cell destruction.

(2) Techniques using air dried smears give, in general, completely useless smears, because this fixation will not immediately stop the autolytic phenomena in the endometrial cells. Thus, in most cases, even the nucleus, the most resistant part of the cell, appeared opaque without any visible nuclear details. These cell changes due to inadequate techniques, which were discussed in a previous symposium, were particularly important if the smears were allowed to dry slowly and remained over a day before staining in a room with warm and moist air.

(3) The degenerative changes are the most marked in smears collected from the vagina, especially in cases of vaginal and/or endometrial infection. This is only due to the cells standing longer in a medium which accentuates the degenerative phenomena beginning in the uterine cavity, so that some endometrial cells can be completely destroyed. Furthermore, the endometrial cells are diluted by the vaginal pool, so that the concentration of the endometrial cells in vaginal smears is very low. Both facts explain the low accuracy of the vaginal smear for the diagnosis of endometrial pathology.

(4) The specimen collected directly from the endometrium by brushing or aspiration, presents the least marked degenerative phenomena, which in many cases are not more than those observed in these cells at biopsy. The endometrial smears also offer the highest concentration of endometrial cells, with a minimum of other cell elements.

(5) The degenerative changes rapidly become more and more pronounced as the collection is done at lower levels, such as the endocervix, ectocervix and vaginal pool. A secondary endometrial infection and/or cervical stenosis preventing a rapid drainage of endometrial cells to the outside, are two important factors for the more marked degeneration phenomena observed in these cases.

CONCLUSIONS

A correct collection, fixation and staining of the smears after the Papanicolaou or the Hematoxylin-Shorr technique produces no evident degenerative changes in the endometrial cells. Only careless preparations or techniques using air dried smears can accentuate the degenerative changes which, in general, are exclusively due to autolytic, enzymatic or necrotic phenomena beginning in the endometrial cells before they are shed. These changes become more and more marked during the passage of the cells from their origin to the site of the collection of the smear. Even with a very careful technique, the degenerative changes of the endometrial cells seen in the usual cytological slides are unavoidable, but they exist at a minimum in the preparations collected directly from the intra-uterine cavity. From this it is concluded that the intra-uterine collection of the smears should be the technique of choice for the cytological diagnosis of endometrial pathology.

DISCUSSION

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

I completely agree with Pundel that air drying of the smear before fixation gives an inadequate

cellular differentiation. In my experience the nuclear detail is not good enough for diagnostic purposes.

Since elsewhere in this journal I have reported a series of carcinomas of the corpus with a high diagnostic accuracy by examination of the vaginal smear, I can hardly agree with Pundel's statement "that the intra-uterine collection of the smears should be the technique of choice." If vaginal smears are properly taken, fixed and stained, the diagnostic accuracy of the vaginal smear in corporal cancer is very close to that of squamous cancer.

I will agree that often the smears from fundal carcinoma have degenerated cells, but in most instances they also have perfectly preserved cancer cells. Whether or not the preserved cells are those which have desquamated most recently is impossible to say definitely, but they probably are. Pundel has clearly presented various reasons for the degenerative changes which occur. I am in complete accord with these. However, I would like to point out that this does not necessarily indicate that all cells are degenerated.

PIERRE HAOUR, Lyon, France:

I have already outlined in this issue the advantages of endometrial aspiration, and I can see that Pundel himself (in comparing his results of cytology done at different levels) concludes that intra-uterine collection is the best method. As stressed by Pundel, in smears from a bleeding uterine cavity, the concentration of endometrial cells is very low in the vaginal pool and very few glandular cells are collected there. It is, therefore, necessary to use the centrifugation technique.

Even when intra-uterine collection is performed and when the smears are correctly fixed, it is not unusual to find the glandular cells with degenerative changes, caused by blood. Working with Kogoj Bokie, we found that if intra-uterine and vaginal cells were mixed with blood or hemolyzed blood and the mixture was kept at a warm temperature or in the refrigerator, degenerative cytoplasmatic or nuclear changes appeared very rapidly except for superficial cells which retained their original pattern for more than a month.

I share the opinion of Pundel concerning the effect of air-drying of endometrial smears. After proper fixation, vaginal smears may be air-dried without any significant loss of detail. In endometrial aspirates, however, air-drying should be avoided.

CLOSING REMARKS

J. PAUL PUNDEL:

There seems to be no disagreement concerning the importance of degenerative changes for the cytological diagnosis of endometrial cancer, with the exception that Ruth Graham said that the diagnostic accuracy of the vaginal smear in corporal cancer is very close to that of squamous cancer of the cervix. Knowing very well the exceptional experience of Ruth Graham in the field of cytodiagnosis, I can only present to her my best compliments for her results which can be due only to her excellent technique. Unfortunately, in my own experience as well as in the experience of others (1-7) the vaginal smear alone has given numerous false negative diagnoses, even after a second study of the smear. It is true that in many cases even the vaginal smear permits the correct detection of endometrial cancer, and I have found several unsuspected corpus cancers in hormonal vaginal smears, but on the other hand, I would like to reemphasize that the endometrial aspiration smear is the technique of choice for the cytological diagnosis of endometrial cancer. This does not mean that endometrial aspiration smears should be taken systematically in every patient, but if the vaginal smear is negative in a patient exhibiting suspicious or merely abnormal uterine bleeding, the possibility of endometrial cancer should not be discarded unless either several endometrial aspiration smears or a complete uterine curettage have been proven to be negative. The danger of the statement of Ruth Graham is (as I had the opportunity to observe in several desperate cases) that the doctor might accept a negative report on an individual vaginal smear as definite proof of the absence of corpus cancer, thus losing precious time if the cytological diagnosis is incorrect. That is what I meant in my paper and I am hopeful that Ruth Graham will now agree with my conclusions

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SHOULD ONE ROUTINELY PERFORM INTRA-UTERINE SMEARS?

HANNS-WERNER BOSCHANN
West-Berlin, Germany

DISADVANTAGES

The following reasons oppose the routine application of intra-uterine smears:

1. Contraindications. Whereas there are no contraindications known for vaginal and cervical cytology, there are the following contraindications for intra-uterine smear preparation:

(a) Purulent endocervicitis, because of the possibility of spreading into the uterine cavity,
(b) diagnosed or suspected pregnancy, because of the possibility of inducing abortion, and
(c) acute adnexitis, as well as subacute adnexitis, because of the possibility of recurrence of the inflammatory reaction.

2. Spectrum of application. Vaginal cytology can be performed by any medical doctor without any specialized gynecological training. The smears can even be prepared by the patient herself (tampon method, centrifugate of the rinsing fluid). However, the introduction of any instrument into the uterine cavity (canula, plastic tube, special brush) requires specialized gynecological training. The size and the position of the uterus must be determined properly, sometimes the endocervix must be dilated, and perforation of the uterus must be avoided. The report by Hecht shows that the danger of perforation is very small if the examination is performed by an experienced person: He did not observe any perforation in a series of 4350 intra-uterine aspirations. When using a small plastic (polyethylene) tube the danger of perforation seems to be rather small.

3. Troublesome procedure. Vaginal and cervical cytology is prepared with disinfected but not necessarily sterile instruments (specula, spatula, cotton swabs, platinum loops) which are available in large quantities for mass screening projects. However, for intra-uterine cytology the same rules apply as for any other intra-uterine surgical procedure which is performed under aseptic conditions. The sterilized instruments (gloves, specula, forceps, dilators, syringes, aspiration canula, plastic tubes or endometrial brushes) require greater expense and are not available in such ample quantities as wooden spatulas. In addition, the intra-uterine procedure is considerably more time-consuming than the procedure of vaginal cytology.

4. Painful. The preparation of vaginal and cervical smears is not painful and is generally not even especially noticed during the routine gynecological examination. The intra-uterine procedure represents a (usually minimal) molestation of the patient. The pain is comparable with that occurring during sounding of the uterus. However, intra-uterine aspirations with a fine plastic tube are usually not painful even in patients with a narrow endocervix.

5. Necessity. The incipient carcinoma on the squamo-columnar junction is usually asymptomatic and usually remains unnoticed when examining with the naked eye or even with the colposcope in cases where the lesion is situated on the endocervix. This is why the routine application of vaginal and cervical cytology is obligatory. However, the corporal carcinoma produces early symptoms, setting aside the fact that corporal carcinomas are much rarer than cervical lesions. We found in our Out-Patient Department of the University of West-Berlin, Moabit Hospital, one invasive cervical carcinoma in 90, one carcinoma in situ in 166, but only one corporal carcinoma in 445 patients. The occurrence of any of the early symptoms of corporal carcinoma (such as previously not present corporal fluor, metrorrhagia, postmenopausal uterine bleeding) is always an indication to perform a curettage with histological examination of the material.

6. Evaluation. Smears from endometrial cells are more difficult to evaluate than vaginal smears since many of the epithelial changes are more characteristic on the squamous epithelium than on the glandular cells. Cytohormonal evaluation from the vaginal smear is much more accurate than from the endometrial cells. One might mention here that the Karyopyknotic Index is only for squamous cells.

ADVANTAGES

Compared with the above disadvantages, one has to consider the following advantages of the intra-uterine procedure:

1. Advantages as Compared with Curettage.

(a) The patient does not need general anesthesia and does not require especially careful treatment after the preparation of the smear since there are no open wounds (unless one had to perform a considerable dilatation of the endocervix).

(b) If properly prepared the intra-uterine smear contains exfoliated cells from the entire surface of the uterine cavity, whereas one obtains only a very small area with an endometrial biopsy. One might even miss a corporal carcinoma located in the tubal corners with a regular complete D & C. Several authors (2, 3, 4) reported about cytologically detected corporal carcinomas in patients in whom the first curettage was negative. In the case reported by Husslein only the fifth curettage brought the histological verification of carcinoma.

(c) Endometrial histology provides a still picture, whereas one can observe the development of an epithelial change over a distinct period of time by means of aspiration cytology. Even after repeated aspirations one does not observe the disturbing changes as inevitably present after curettage.

(d) Often one finds enough material for cytological examination even in those cases in which the curettage does not yield sufficient material. Therefore, in those cases we advise smearing the material on a glass slide and fixing and staining it according to the Papanicolaou technique, especially if pre-surgical intra-uterine smears were not performed.

2. Advantages as Compared with Vaginal Cytology. Vaginal, cervical and endocervical cytology in the detection of corporal carcinoma have only a diagnostic accuracy of 50-60%. In our material of 96 cytologically examined corporal carcinomas, vaginal and cervical smears were positive in only 31 cases (32%), endocervical smears were positive in 59 cases (61%) and endometrial aspirations were positive in 83 cases (86%). Similar results were published by Wied (10) on the first 32 cases on this particular series. Only the preparation of the smears from the uterine cavity results in reliable findings (2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17).

3. Advantages as Compared with Other Gynecological Methods of Examination. We found an adenocarcinoma in a routinely performed intra-uterine smear in a 46 year old patient (with the clinical diagnosis of "chronic adnexitis"). During surgery we found an ovarian carcinoma which had penetrated into the uterine cavity. According to this experience one should actually demand that an intra-uterine smear belongs to every gynecological cancer detection examination. In addition to that, it is rather easy to distinguish these three endometrial cell types in cases of postmenopausal uterine bleeding: senile endometrium, glandular hyperplasia and carcinoma. Especially in cases of uterine bleeding the preparation of the intra-uterine smears is rather painless since the endocervix is usually open.

If one evaluates the disadvantages and advantages of the technique, one might agree principally that the intra-uterine method should be performed routinely. However, one may recommend it only in distinct indications, and routine application does not seem advisable. Intra-uterine cytology should be prepared by the specialized gynecologist and clinician in every case of clinical suspicion of endometrial, tubal and ovarian carcinoma (i.e., in metrorrhagia, and postmenopausal bleeding) if:

1. there are contraindications to perform a curettage (e.g., impossibility of performing general anesthesia),
2. the patient does not permit a curettage,
3. the curettage does not yield sufficient material. (In these cases we advise preparing the smear directly from the material.)

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WARREN R. LANG

Philadelphia, Pennsylvania, U.S.A.

Intra-uterine smears are of more than academic interest in the diagnostic evaluation of the mature, non-pregnant, non-infected female.

A preliminary pelvic examination should be performed to determine the position of the uterus, the presence of pregnancy or the possibility of parametrial inflammation. The latter two states obviously contraindicate the method. The technique is a simple, safe office procedure. A nasopharyngeal cannula, with a Luer-Lock syringe, or the newer Milex cannula can be employed to enter the uterine cavity. Polyethylene tubing and a brush technique have also been described. If the cervical os is stenotic, a tenaculum is applied and the opening enlarged with a uterine dressing forceps.

Since the endometrial aspiration technique procures cells directly from the endometrial cavity, the cells obtained are usually less degenerated than the corresponding cells in the vaginal smear. In addition, the cells are more concentrated and represent a more efficient sampling of the area. Wied has called attention to the importance of seeking cells as near as possible to the area under question [1]; the closer the instrument is to the endometrium the more apt we are to secure endometrial and ovarian cells.

The endometrial smear technique can be incorporated without difficulty into routine smear taking in both symptomatic and asymptomatic women. In the presence of irregular bleeding and postmenopausal bleeding the smear may serve to alert the clinician to the presence of cancer. It should be recalled that even the curette may miss endometrial cancer and of course can furnish no information concerning tubal or ovarian carcinoma. In these two instances the smear may be the only guidepost to further investigation. In presumably healthy women, incipient malignancy may be detected. The smear is also of value in benign states such as hyperplasia, polyps and infertility. From the viewpoint of the cytologist routine aspiration smears furnish more cells, so that a more complete evaluation can be made; in addition, only by the study of many normal cells can the abnormal ones be detected with certainty.

We have experienced no adverse effects with routine intra-uterine smears and believe the few extra minutes required to perform them are well worth the added information gained.

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EDMUND SCHÜLLER

Vienna, Austria

The endometrial smear is not sufficiently practical for routine usage. The technique is not simple, often causes considerable discomfort and even danger to the patient and must be performed under sterile conditions. It seems to me nonsense in prefering a dangerous procedure which can only give a preliminary diagnosis. In cases where there is any suspicion of a fundal lesion, such as irregular or menopausal bleedings, one should prefer introduction of a curette, in order to obtain tissue for histological examination and, therefore, a final diagnosis.

DISCUSSION

WERNER BICKENBACH and HANS JÜRGEN SOOST, Münich, Germany:

Intra-uterine smears, in our opinion, may become necessary for diagnostic purposes in cases where the patient refuses curettage or where, for some reason or other, curettage cannot be done.

There is no doubt that the early diagnosis of fundal carcinoma can be improved by intra-uterine cell aspiration. In clinically suspicious cases, aspiration from the uterine cavity can be done immediately prior to curettage if it is thought necessary. In cases of cancer of the uterine body with negative histology, or of tubal cancer, a suspicious smear would give rise to an even more intensive search, or at least one would keep the patient under very close observation.

However, we do not recommend that intra-uterine smears be done routinely in the search for intra-uterine cancer. Since corporal carcinoma is much less frequent than cervical carcinoma and since the former usually presents early symptoms and grows slowly, the technical demand does not seem justified to us, especially as the procedure, in untrained hands, may be dangerous to the patient.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

All three authors agree that the technique for intra-uterine smears is more time-consuming and difficult, and Schüller is very emphatic that they should not be used as a routine method. In contrast to this, the American contributor to this symposium feels that the few extra minutes of additional time are well spent for the added information gained. We have been so discouraged by our high percentage of false negatives in endometrial carcinomas diagnosed by vaginal smears only, that we have recommended this method as a matter of routine in all menopausal women. However, we have found a considerable resistance to it, even by the experienced gynecologist, who hates to subject his patients to any type of discomfort.

F. A. IKLÉ, St. Gallen, Switzerland:

The findings of our last 374 aspirations have been collected. Three-hundred and fifteen smears have been interpreted as negative and 18 (4.81%) as negative with the remark that carcinoma of the endometrium could not be excluded and further controls or curettage seemed advisable. Sixteen (4.28%) were positive for carcinoma and in 25 (6.65%) cases the material was too scanty to allow examination. Our earlier experience (1) has so far been confirmed.

By no means should the method be compared with curettage. But until now there is no better or easier substitute for this office procedure. The accuracy is judged differently. According to Hecht and Papanicolaou it goes well above 90% (2) (3). Our own results are not as good (85%) but can be improved by further training. On the other hand, the method allowed:

1. the diagnosis from a few asymptomatic cases of endometrial carcinomas (cases without indication for curettage).
2. the exclusion of unnecessary curettage in a high percentage of cases with benign irregular or menopausal uterine bleeding; cases with conventionally accepted indication for curettage. (The 315 negative cases have been followed up for from 1 to 2 years. Until now they have remained asymptomatic.)
3. some information about the benign changes of the endometrium as hypo- or hyperplasia. (Using P.A.S. staining, deficient progestional changes can also be detected.)

We had a case of polyposis of the endometrium with simultaneous adenocarcinoma which was missed by the aspiration technique, but in this case the result of the first curettage was also negative. Half a year later, the correct diagnosis was confirmed by aspiration smears as well as by curettage.

In spite of this, we would not like to lose the intra-uterine aspiration smears for the diagnostic evaluation of the endometrium.

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LUBA A. TURNBULL, Montreal, Canada:

If the vaginal or cervical smear is very simple to make and it could be done by every trained person, the endometrial smear should be done by a medical doctor or better by a gynecologist, after a routine pelvic examination with sterile instruments to prevent an infection or even an uterine perforation. Of course this manipulation takes longer than the usual cervical smear, but it is correct that cells taken directly from the endometrium seem comparatively normal as compared to those in vaginal smears.

We have studied smears taken from 400 women where two cervical and one endometrial smear by intra-uterine aspirating cannula were taken.

We have had two cases from these 400 with early pregnancy, where the intra-uterine smears were taken and a mistaken diagnosis was made with the recommendation of curettage. In these 400 patients, however, we did not diagnose one case of endometrial carcinoma.

A few years ago we had one case of a 58 year old patient with symptoms of spotting 8 years after menopause. This patient came to us after having had a cervical smear with negative results done in an-

other country. One of our staff performed an intra-uterine aspiration smear that was positive for adenocarcinoma. Curettage was performed in our hospital and the pathology report showed an early adenocarcinoma of the endometrium.

I do not agree with Schuller, who described the endometrial smear as "dangerous procedure" --if one prefers to introduce the curette, it could be more dangerous than a cannula or a brush.

My opinion in this question is that it is an individual matter, consisting of what you consider best for a prompt diagnosis.

It is necessary to do endometrial smears in women who have some symptoms of vaginal discharge or spotting and similarly those who have a prolonged menopause.

CLOSING REMARKS

HANNS-WERNER BOSCHANN:

I am glad to find agreement that endometrial cytology, although not as obligatory a routine method as vaginal cytology, can be an additional diagnostic help if other methods fail or cannot be applied in cases suspicious for endometrial carcinoma.

WARREN R. LANG:

I suppose that one's viewpoint on the subject of routine intra-uterine smears is the product of many factors, including whether or not one is a cytologist, a gynecologist or a combination of the two. In addition, past teaching and experience come into play. The crux of the discussion probably centers around the word "routine."

It is true that intra-uterine smears must be taken by a trained and interested physician. The contraindications mentioned previously should be observed. I do not believe that otherwise the procedure is dangerous. Curettage is a more specific method of diagnosis and if the clinical symptomatology suggests the necessity for curettage, this should be done – no matter what the intra-uterine smear indicates. In other words, the smear is not a substitute for curettage even though the former on occasion will discover asymptomatic lesions.

EDMUND SCHULLER:

The arguments put forward for taking intra-uterine smears could not convince me. Carcinoma of the uterine body practically always causes symptoms at a time when it can still be treated successfully, so that one can abandon taking routine intra-uterine smears from women who show no symptoms. If there is any symptom present, even if it should seem quite insignificant, a curettage must be done as the most reliable diagnostic measure, and, therefore, there is no point in taking intra-uterine smears prior to this.

The curettage must be carried out by a competent gynecologist using general anaesthesia. If this is the case, the histologist will always be able to make the correct and final diagnosis.

A CLEARING TECHNIQUE IN ENDOMETRIAL CYTOLOGY

B. CORNELIS HOPMAN
Miami, Florida, U.S.A.

The cytologic diagnosis of cervical cancer has progressed to such a high degree of accuracy (in these days) that the cervical smear has become a powerful tool in the field of gynecologic diagnosis. Its simplicity, speed, high dependability, extensive information yielded and the relatively low cost make it one of the most frequently performed tests, almost a necessity before any gynecological examination or treatment is started.

In testing for gynecological cancer it soon became apparent, however, that although the results for cervical cancer were highly accurate and dependable, those for endometrial cancer lacked the same exactness. According to Hecht (1-4) up to about 50 per cent were missed.

There are several reasons for this shortcoming. To begin with, one does not encounter endometrial cells in the usual cervical smear. They appear only in cases of menstruation, endometrial hyperplasia and endometrial cancer. Second, endometrial cancer mostly occurs in women during the postmenopausal years (beyond fifty years) when some degree of cervical stenosis may have developed impeding the descent of exfoliated cells from the uterine cavity into the cervix and vagina. Furthermore, the cells may take so long to reach the cervix that by then they have degenerated beyond recognition. Third, the character of the endometrial cells makes it difficult to recognize them from cancer cells. Endometrial cells are small cells not much larger than leukocytes. The cell is already completely differentiated when first formed, contains a small nucleus surrounded by only a very small rim of cytoplasm in which vacuoles are rarely visible. The significant differences which distinguish normal from malignant squamous cells very often are lacking or difficult to recognize when one examines endometrial cells. The rim of cytoplasm in cancer cells is sometimes larger, vacuolated, and the nucleus is hyperchromatic with irregularly distributed and coarsely clumped chromatin. Of help also is a visible nucleolus of irregular size and shape. The cells may be present in small groups. Notwithstanding all these characteristics of malignant cells, their differentiation, especially from the equally enlarged cells of the hyperplastic endometrium, often remains difficult.

In recent years the introduction of the aspiration techniques by Papanicolaou (5) and Hecht (1-4) has contributed to a more accurate cytologic diagnosis of endometrial cancer.

A very important obstacle in the diagnosis of endometrial cancer, regardless of the manner in which the cells were secured, is, in our experience, the presence of red blood corpuscles and mucus in sufficient quantities to cover and obscure the cells, making a diagnosis almost impossible (Fig. 1). We believe we have made an improvement in smear preparation and have overcome this part of the difficulty of endometrial diagnosis. Then there followed better understanding of the cytologic characteristics of the normal cells in the different stages of the hormonal cycle, as well as of hyperplastic endometrial cells and of malignant cells.

Türk's (6) fluid, known since the beginning of the century, consists of dilute acetic acid which hemolyzes the red blood corpuscles, so that leukocytes contained in the fluid can be recognized when stained by the gentian violet. Experimentation with acetic acid in different concentrations revealed that its hemolytic property could be applied successfully to cytologic smears. It was used after fixation with alcohol-ether but before Papanicolaou staining. Different concentrations were tried from glacial acetic acid down to 1 per cent. In the original paper (7) glacial acetic acid applied for 15 minutes was described as the best clearing method. This is correct and is still used by us when much blood is present.

This work was supported by a research grant from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

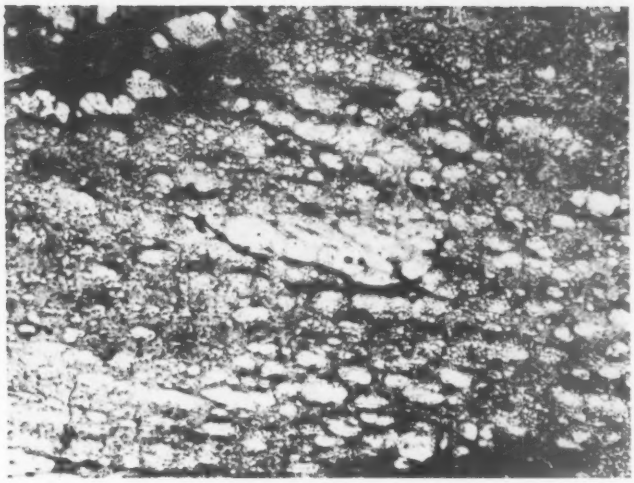

Fig. 1. Presence of red blood corpuscles in the endometrial smear obscures the cells, making a diagnosis impossible.

Fig. 2. A slide is placed in a trough of paraffin in a Petri dish filled with acetic acid.

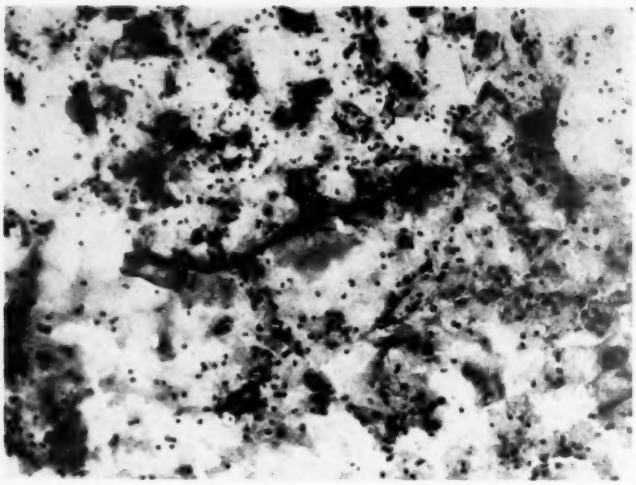

Fig. 3. Effect of clearing technique in cervical smear.

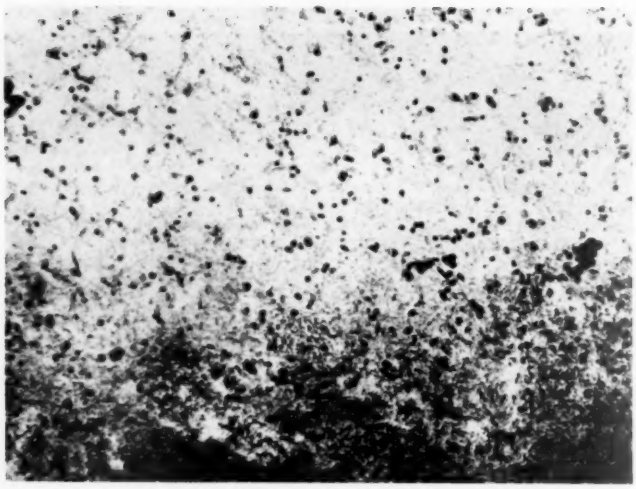

Fig. 4. Effect of clearing technique in endometrial smear. Cancer cells almost invisible in the blood, low in the figure to the right of the center, better visible on the border line to the right of the center, best visible in the acid part somewhat higher up, left of the center.

However, further experience has shown that acetic acid influences the slide in two ways. First, it is a known fact that selectivity of hematoxylin for cell nuclei is increased by the presence of an acid pH (8). Although the bathing of the slide in acid does not influence the pH of the hematoxylin of the Papanicolaou stain because the slide is first rinsed in water to remove the acid, still there is some increase of nuclear staining. The difference is only slight and when the slide is not over-stained it will scarcely be visible, but if there is already some over-staining it makes the nuclei too dark. The reverse can be observed in cells in an alkaline medium, as in chronic infection, especially trichomonas infection. These tend to make the Papanicolaou stain paler and the nuclear stain inadequate, now and then causing false negative cancer diagnoses.

Another fact we observed was that high concentrations of acid in non-vaginal smears tends to remove some of the cells, probably due to loss of some of the mucinous material needed for adhesion of the cell to the glass. This was no problem in cervical smears but happened mostly in urine smears which are easily removed from the slides.

These experiences induced us to review the concentrations of acetic acid and we now use 10 per cent acetic acid for five minutes as a routine for most of the slides. For large amounts of blood the concentration used is higher. For urine smears the concentration used is lower. With these concentrations enough blood is cleared to make the cells easily visible, and the intensity of the nuclear staining is not influenced, nor is the adhesion of the cells to the slides.

A convincing demonstration of the clearing property of acetic acid can be obtained by a method formerly used to show the action of DNA-ose on DNA (9). In a Petri dish previously filled with paraffin, a trough is made in the wax and filled with acetic acid. A slide is placed in it on its longitudinal edge so that only one half is immersed (Fig. 2). When the slide is stained and examined the effect of the acid can be seen along the line where the halves meet (Figs. 3, 4).

The influence of acetic acid on desoxyribonucleic acid of the nucleus is nil. This can easily be examined by the following test. Smear some DNA evenly over a slide. Put the slide in the paraffin trough described above, filled with 10 per cent acetic acid. Stain with hematoxylin. Both halves are stained to exactly the same intensity. There is no difference in adhesion. The action of glacial acetic acid on blood is employed in the Teichmann method (10). Blood mixed with glacial acid is heated. The hematin formed from the hemoglobin treated with hydrochloric acid forms brownish rhombic crystals, lying singly or in crosses.

Spectrophotometric analysis of blood with saline and similar quantities of blood with diluted acetic acid show differences which are in accord with the experimental clearing reactions. Acetic acid blood has its maximal absorption at 400 mμ, the saline-blood at 450 mμ. The acetic acid blood curve is on a much higher level and this allows greater light transmission (Fig. 5).

Although this report describes a clearing technique for endometrial diagnosis, it may be equally applied for cytologic smear preparations from any other organ.

SUMMARY

A clearing technique using diluted acetic acid will clear endometrial smears of blood and mucus, making the cells more readily visible, thus improving cytologic diagnosis. In dilute solutions, acting for five minutes, the acetic acid does not influence the staining of the cell nucleus nor the adhesion of the cells to the slides. In general, 10 per cent acetic acid is used; for much blood, higher percentages are required and for urine lower percentages. The reaction of this technique can be seen when half of a slide is dipped in a trough of paraffin filled with acetic acid so that the effect of the acid can be compared with the untreated half of the slide. The technique is applied after fixation of the smear and before application of the Papanicolaou staining. Experiments with DNA smears on slides confirmed the above described experiences. Spectrophotometric analyses of blood with and without acetic acid are in accord with the observed clearing reactions. This technique is also useful for cytology of other organs.

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SPECTROPHOTOMETRIC CURVE

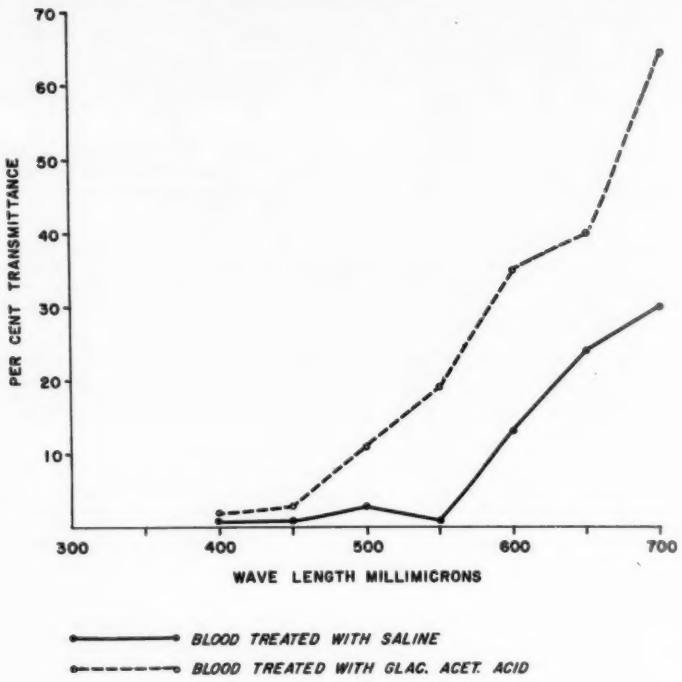

Fig. 5

DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

In a comparative study with my co-workers Drs. Kur and Fixson, we added glacial acetic acid to the routinely used fixation fluid (ether, 95% alcohol).

A. Three parts of glacial acetic acid + 97 parts of ether-alcohol (60 minutes):
   erythrocytes: still shadowy, some well-preserved, e.g., at the edge of cell sheets
   nuclear structures of epithelial cells: very well-preserved
   cytoplasm: very well-preserved

B. Six parts of glacial acetic acid + 94 parts of ether-alcohol (60 minutes):
   erythrocytes: some shadowy, some totally disappeared, no preserved red corpusules
   nuclear structures of epithelial cells: well-preserved
   cytoplasm: well-preserved
   Hormonal diagnosis possible, even if the smear had been taken during uterine bleeding.

C. Ten parts of glacial acetic acid + 90 parts of ether-alcohol (20 minutes):
   erythrocytes: totally disappeared, no shadows left
   nuclear structures: sufficiently preserved
   cytoplasm: partially destroyed

One hour's fixation with six parts of glacial acetic acid gave better results than twenty minutes fixation with ten parts of glacial acetic acid: The smear was clearer, and a greater number of erythrocytes had disappeared. We did not see the removal of cells from our smears, because we applied simultaneously glacial acetic acid and ether-alcohol in a mixture. By successive application of the components we saw the removal of cells described by Hopman.

JEAN de BRUX, Paris, France:

I am personally grateful to Hopman for his very interesting scientific paper. In my laboratory we have used the Türk's fluid for red blood cell hemolyzation for a long time. We will try Hopman's concentration in acetic acid, but I want to ask him how he can make the cells adhere sufficiently to the slide in cases of pleural and peritoneal effusions. Hopman said "for urine smears the concentration is lower." What is this concentration?

GUILLERMO TERZANO, Buenos Aires, Argentina:

Because there are false negative smears in cases of endometrial carcinoma, as Hopman points out so well, any technique to improve the diagnostic accuracy is enthusiastically received.

Acetic acid for clearing has been used in our laboratory. Sometimes the expected lysis of the erythrocytes was not very satisfactory. However, usually the dipping of the slides in acetic solution proved to be effective, with no appreciable changes in the staining reaction of the smears if stained according to Papanicolaou's technique. Nevertheless, we prefer spreading the specimens (endometrial aspirations) on two, three or more slides, making a very thin film on each. Doing so, blood cannot be blamed for a false negative report.

CLOSING REMARKS

B. CORNELIS HOPMAN:

In reply to the discussion by Dr. Boschann:

I agree that 10 per cent glacial acetic acid will cause the disappearance of erythrocytes with preservation of the nuclear structures. I did not apply a glacial acetic acid and ether-alcohol mixture simultaneously as a routine because it interfered with the pH of the stain. Glacial acetic acid is not used routinely but only when necessary to remove blood.

In reply to the discussion by Dr. de Brux:

When the slides in cases of pleural and peritoneal effusion and urine contain enough cells, a ten per cent acetic acid solution will not cause a removal of cells worth mentioning. If there are only a few cells on the slides, the interpretation will be difficult. The application of 5 to 10 per cent acetic acid solution will not be harmful.

In reply to the discussion by Dr. Terzano:

I agree that endometrial aspirations made as a very thin film on slides are theoretically preferable to acetic acid treatment but are not received as such in the laboratories.

Symposium C

ENDOMETRIAL CYTOLOGY IN ENDOCRINOLOGY

ENDOMETRIAL CYTOLOGY DURING THE FOLLICULAR PHASE OF THE MENSTRUAL CYCLE

HANNS-WERNER BOSCHANN
West-Berlin, Germany

The present report is based on 133 cytological examinations of endometrial cavities of uteri which were removed during the proliferative phase of the cycle. Prior to hysterectomy a smear was prepared by means of the endometrial brush technique or the aspiration method. After surgery the uterus was cut open and direct smears were prepared from the endometrial area which was later examined histologically. These resulting smears and sections were compared. Only if the histological section exhibited definite evidence of proliferation was the cytological smear evaluated for this report. These examinations resulted in the following findings (also see the paper in this edition entitled "Cytomorphology of the Normal Endometrium"):

The glandular cells during the proliferative phase of the cycle exhibit an oval, sharply defined nucleus with finely granulated chromatin. The nucleus is usually in the center of the slender cell which is generally so narrow that the nucleus bends the cell outwards. The chromatin is dense during the proliferative phase and loosens up as this phase progresses. The nucleus becomes rounder. After the 10th day one finds cytoplasmic vacuoles which are, therefore, not significant for the secretory phase. Often one finds mitoses in the nuclei. Also, the chromatin of the stroma cells is rather dense during the beginning of the proliferative phase; sometimes spindle-shaped forms can be seen.

If the above cells are found in the secretory phase of the cycle, one usually deals with either glandular or stromal cells from the basalis which participates only to a small extent in the cyclic changes, thus, falsely giving the impression of a proliferative phase. Or it may be that one deals with an anovulatory cycle or beginning glandular hyperplasia respectively. The characteristic cytological pattern for follicular persistency in the absence of corpus luteum hormonal stimulation is as follows: hyperplastic glandular cells with abundant cytoplasm which may be extremely elongated; the chromatin content is slightly increased and there might be a variance in nuclear size and form. One can differentiate these cells with their regular chromatin distribution from cells from endometrial carcinoma. Concerning the changes of cellular size also see the paper in this edition entitled "Cytometry of Normal and Abnormal Endometrial Cells."

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CLARICE do AMARAL FERREIRA
Rio de Janeiro, Brazil

Since the knowledge of Romberg's work on hormonal endometrial cytology (1), we have been interested in this study, especially to provide some aid in hormonal sterility problems. We have studied more than 250 cases with stained smears obtained from endometrial aspiration or brushing, from normal and disturbed ovulatory cycles (2). Now we are examining this material using vital staining with toluidin blue. With this method one obtains a pronounced blue staining of the nuclear chromatin and a pink staining of the glandular cells.

In about 100 cases we have studied the glycogen content of the endometrial cells by the MacManus method (3). In normal ovulatory patients we have found, at the sixth to eighth day of the cycle, a compact stroma grouping of cells, with regular nuclei, visible chromatin and scanty cytoplasm. Shortly before the fourteenth day of the cycle an increase of pyknosis is seen.

We were not able to characterize the phases by changes of the stromal cells, like Romberg. It seems easier to us to characterize changes in the epithelial cells, especially in the glandular cells. The epithelial cells at the seventh or eight day of the cycle are round, single or in little groups, sometimes vacuolated, with great and regular nuclei, commonly eccentric (Fig. 1). These cells begin to assume an elongated shape, with basal nuclei, as they reach the ovulation date. Sometimes in the ovulatory period we can see some large groups of cells with central nuclei; the groups seen in the transversal sense give the aspect of "honeycomb" (Fig. 2). One frequently finds (in any phase) a predominant cell type which is characteristic of this phase, but also in addition to these, some cells from a later or an earlier stage. When one obtains cytological material from a large area of endometrium, one can imagine that the endometrial changes are not as uniform as the histological sections show. Usually in some areas local vascular conditions, or possibly hormonal irresponsiveness, do not permit the regular cytologic changes.

Considering that the MacManus staining technique is one of the best for glycogen and that cells staining red usually contain glycogen in great amounts, we can say that in the first week there is practically no glycogen in the cells; it appears first in the cytoplasm of the stromal cells, then in the epithelial cells and increases as the endometrium reaches the ovulatory phase and continues after that. It seems that the presence of glycogen is correlated with estrogenic activity.

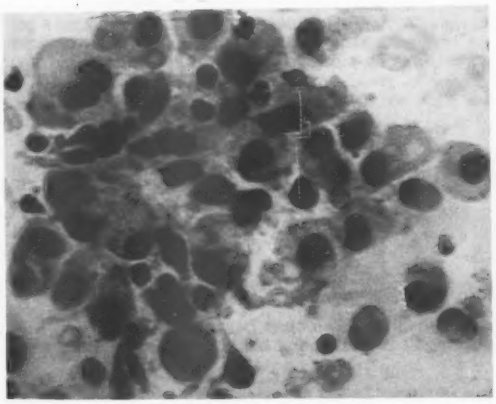

Fig. 1

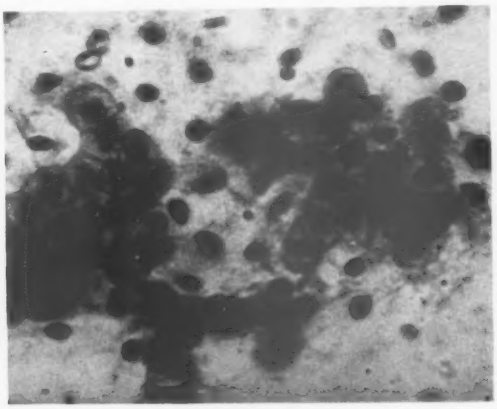

Fig. 2

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HANNAH PETERS
Bombay, India

Ample material for cytological study can be aspirated from the endometrial cavity at any time of the cycle. Considerable amounts of tissue are desquamated during the late secretory and menstrual phases, and large bits of endometrial epithelium are found in the early as well as the middle part of the cycle. Two types of epithelial cells are seen in the normal endometrial smear. The stroma cells are usually found in loose groups (Fig. 1). They show a marked variation in the size and staining quality of their nuclei and are characterized by a lack of nuclear uniformity. The stromal cells show no characteristic cyclic changes. The columnar cells, however, which are seen singly or in large, rather dense cell aggregates and groups, show changes at the various times of the cycle, which are marked and constant. The columnar cells are uniform in appearance. The nuclei vary in their size and characteristics according to the time of the cycle and the state of the endometrium, but on a specific slide and within the same group they exhibit a marked uniformity. In the early part of the proliferative phase the columnar cells have little cytoplasm, the nucleus seems to fill the whole cell. The distinct nuclear membrane appears prominent due to the uniformly pale staining nucleus in which a small, dot-like nucleolus usually stands out (Fig. 2). This "inactive" looking nucleus is characteristic for the early proliferative phase. As the glandular epithelium of the endometrium becomes more active in the later stages of the follicular phase, the nuclei of the columnar cells in the smear change their appearance. Cells with pale nuclei become rare. In the middle and late follicular phase, the nuclei of the columnar cells stain darker and show chromatin stippling and clumping (Fig. 3). Throughout the follicular phase the presence in the cytoplasm of considerable amounts of ribonucleic acid can be demonstrated (methyl green-pyronine staining and digestion with ribonuclease as control).

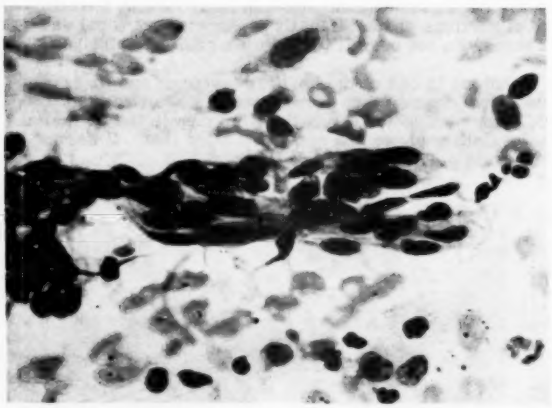

Fig. 1. Stroma cells vary in size, shape and staining quality. Their variation within the same cell group is in contrast to the marked uniformity of the columnar cells. (Hannah Peters)

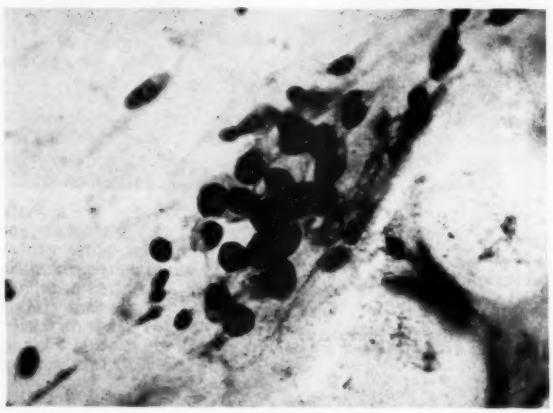

Fig. 2. Columnar cells in the early proliferative phase show a uniformly pale staining nucleus with a distinct, dot-like nucleolus ("inactive nuclei"). (Hannah Peters)

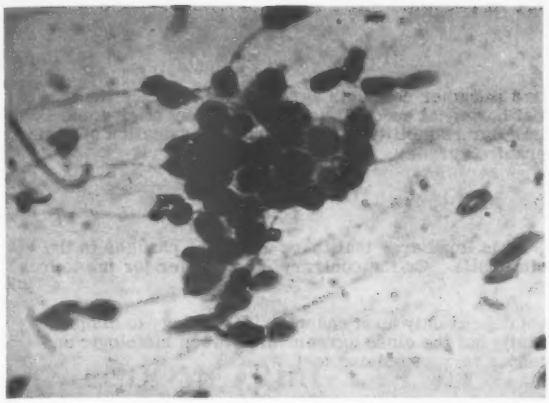

Fig. 3. Endometrial cells in the late proliferative phase are characterized by large nuclei with deep staining, granular chromatin material. (Hannah Peters).

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

When an intra-uterine smear is taken during the follicular phase, one must expect to find epithelial and glandular cells from the functional region, and stroma cells. These cells are difficult to differentiate when they appear singly. Cells from the basal layer are relatively rare. Our observations regarding the shape and the changes of the glandular cells agree with those of the other authors. The nuclei of the stroma cells often have irregular shapes and peripheries. In general they appear darker. However, they do not show granular chromatin structures. The difference in size of the nuclei is more marked than in the glandular cells. Certain irregularities in the shape of the nucleus are also often found in epithelial cells from the surface of the endometrium.

MANUEL GALBIS, Valencia, Spain:

With the histochemical study of the normal endometrium (see "Cytochemistry of Normal and Abnormal Endometrium") we have also investigated the same reactions. (PAS, methyl green-pyronine and alkaline phosphatase) in the exfoliated endometrial cells obtained by endometrial aspiration. We have directed our attention to the histochemical aspect more than to the morphological one.

In the early proliferative phase we have been unable to demonstrate the presence of glycogen in the scanty cytoplasm of glandular cells. The distribution of the pyroninophilic stain and the alkaline phosphatase shows marked irregularity. In the late proliferative phase there is some glycogen in an occasional cell but its distribution is irregular and exhibits no definite pattern. The same is true with the pyroninophilic stain and the alkaline phosphatase. As opposed to the uniformity seen in tissues, the exfoliated cells stain poorly and the distribution of the reaction varies greatly. Our results are not identical to the ones seen by Ferreira and Peters.

The nuclear structure, as evidenced by the methyl green stain exhibits variations like the ones described by Hannah Peters. The nucleoli are larger and it is difficult to see a pyroninophilic reaction.

The histochemical reactions in the cytoplasm are so variable and inconsistent that no definite conclusions can be drawn.

JOSÉ MARIA MEZZADRA and GUILLERMO TERZANO, Buenos Aires, Argentina:

Well-preserved endometrial (stromal and columnar epithelial) cells can be studied and evaluated in smears fixed and stained according to Papanicolaou's technique.

Noticeable changes in the stroma cells were not observed when comparing smears of the follicular phase with smears of the progestational phase. We have observed the morphological differences described for the epithelial columnar cells in smears obtained before or after ovulation in a normal biphasic cycle, but we cannot consider ourselves qualified to recognize these endometrial clusters (obtained by endometrial aspiration) as a reliable test in evaluating progestational effect upon the endometrium. In other words, we cannot yet depend upon endometrial cytology to diagnose ovulation, especially when we are dealing with women complaining of sterility. Further research will perhaps discover specific signs in the desquamated cells.

It is our opinion that endometrial aspiration provides interesting material for cytological research; in practice it is a useful test when used along with seriate vaginal smears, basal body temperature and crystallization of the cervical mucus, in dealing with problems related to functional sterility.

CLOSING REMARKS

HANNS-WERNER BOSCHANN:

According to the discussion remarks there is rather complete agreement among the findings of different investigators, as well as in the opinion, that hormonal endometrial cytology may offer some points of scientific value, but by no means is of a similar reliability as the hormonal evaluation of vaginal squamous epithelial cytology and basal body temperature curves.

CLARICE do AMARAL FERREIRA:

Bickenbach and Soost are of the opinion, as is Romberg, that there are more changes in the shape of stromal cells than of glandular and epithelial cells. On the contrary, it is easier for me to look for cellular changes in epithelial cells.

As Mezzadra and Terzano, we also do not depend only upon endometrial cytology to diagnose ovulation (we never depend upon only one test for that), but the close agreement between histologic and cytologic diagnoses makes endometrial cytology study a very promising test.

ENDOMETRIAL CYTOLOGY DURING THE LUTEAL PHASE OF THE MENSTRUAL CYCLE

HANNS-WERNER BOSCHANN
West-Berlin, Germany

The present paper is based on experiences with 103 cytological examinations of endometrial cytology with the aspiration or brush technique during the luteal phase of the normal menstrual cycle. The same day the cytological examination was performed the patients underwent hysterectomy (usually for myoma). Another smear was prepared from the open uterine cavity after surgery. The same area from which the postoperative cytological specimen was taken was then examined histologically. From these studies the endometrial cytological changes during the luteal phase could be described as follows:

The glandular cells during the proliferative phase are slender and are bent outwards by their nuclei. During the luteal phase of the cycle the glandular cells become wider (sometimes only at one end of the cell, thus showing the shape of a champagne glass), later more plump and finally nearly cubical. The cytoplasm degenerates so easily that one encounters practically only free nuclei in the smear. These changes must be understood in connection with the secretory changes. Mitoses are extremely rare.

The stroma cells show a gain in cytoplasmic substance and marked cellular borders (predecidual changes). The cells exhibit postovulatory shrinkage of the nuclear and cellular areas, which is followed by an increase in the size around the 18th to 20th day of the cycle (see paper in this edition entitled "Cytometry of Normal and Abnormal Endometrial Cells").

If during the secretory phase of the cycle, one finds glandular cells with cytological signs of the proliferative phase (marked chromatin content, mitoses, oval to spindle-shaped nuclear form), one usually deals either with cellular elements from the basalis (which usually does not participate in the cyclic changes) or an anovulatory cycle or beginning follicular persistency (with development of glandular hyperplasia) respectively. However, because of the possible sources of error involved (especially the difficult evaluation of the cellular elements of the basalis), we consider it more advantagous to rely on vaginal cytology, basal body temperature measurements and endometrial punch biopsies when dealing with problems of sterility (especially as to the presence or absence of anovulatory cycles), and not to rely on endometrial cytology.

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CLARICE do AMARAL FERREIRA
Rio de Janeiro, Brazil

In the normal ovulatory cycle, after ovulation, one notices an increase of pyknosis in the stroma, and the nuclei appear to be closer, until the end of the cycle. The epithelial cells have translucid cytoplasm and appear in groups, the nuclei begin to shrink and we more frequently see cellular groups exhibiting the "honeycomb" pattern (Fig. 1). Toward the end of the menstrual phase the cytoplasm is disintegrated, and one sees only nuclei surrounded with irregular masses of clear, vanishing cytoplasm (Fig. 2).

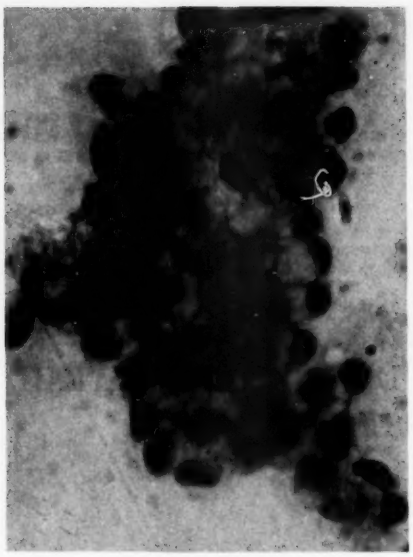

Fig. 1

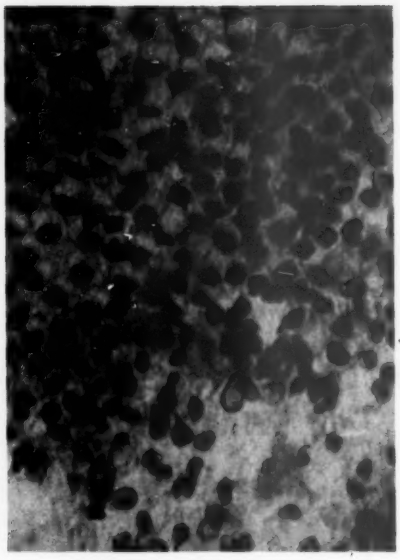

Fig. 2

Until the twenty-second or twenty-fifth day of the cycle one sees, with the MacManus stain, red cells appearing rich in glycogen and mucus: Towards the end of the cycle this substance leaves the cells and we see clouds of pink material in the smear.

In cases of mixed endometrium one readily can distinguish the different cellular patterns. Groups of proliferative cells lie next to groups of secretory cells.

HANNAH PETERS
Bombay, India

Endometrial aspiration during the luteal phase yields a considerable quantity of tissue which can be easily smeared and examined. The changes that take place in the columnar cells during this phase of the cycle are first recognized in the cytoplasm of the cells. The cell enlarges mainly due to an increase in the amount of cytoplasm. In the earliest part of the luteal phase single, large cytoplasmic vacuoles become prominent. They can easily be recognized under low power. Whereas the nuclei in the columnar cell groups during the follicular phase are closely packed and seem to leave little room between each other, the cell groups soon after ovulation take on a lacy appearance as the nuclei of the cells seem to be pushed apart by empty spaces (Fig. 1). Under higher magnification, the vacuoles are clearly seen (Fig. 2). They correspond to the vacuoles seen in endometrial sections (Fig. 3). As the secretory phase progresses, the cytoplasmic changes disappear and nuclear changes become characteristic. Some nuclei become small, shrunken and black in appearance. No nuclear details are left in these "burnt-out" nuclei. Other nuclei at this stage show just a membrane around a faintly stained center in which neither chromatin details nor nucleolus is recognized. The nucleus then looks like an empty shell. The "burnt-out" and the "shell-like" nuclei are characteristic of nuclear degeneration in the late secretory phase. Cytoplasmic ribonucleic acid, characteristically found throughout the follicular stage decreases rapidly after ovulation and is absent in the later stages of the luteal phase.

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

In intra-uterine smears taken during the luteal phase, we noticed, similarly to Hannah Peters, first of all the enlargement of the cytoplasm which the nuclei do not appear to share. The cytoplasm is usually badly defined, irregular in shape, drawn out and may be vacuolized. Frequently the nuclei are completely denuded. The larger, lighter stained as well as the shrunken, darker stained nuclei show a loss of fine structures.

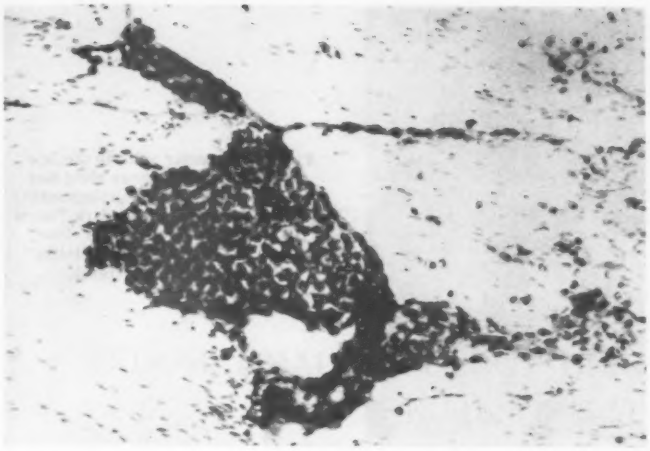

Fig. 1. Group of columnar cells in the early secretory phase. The large cytoplasmic vacuoles give the group a lacy appearance. (Dr. Peters).

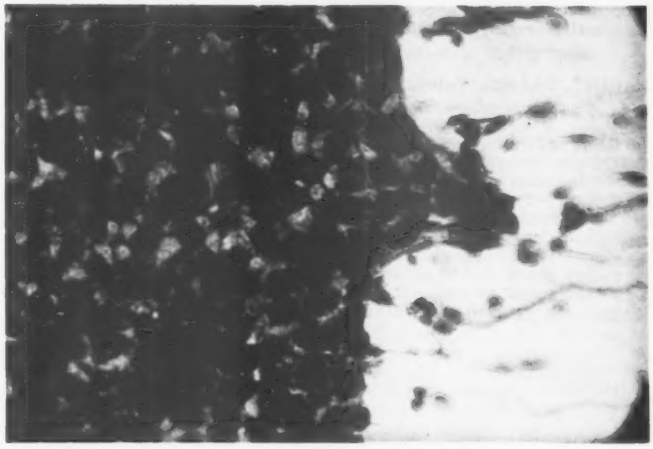

Fig. 2. Columnar cells in the early secretory phase show large, single cytoplasmic vacuoles. (Dr. Peters).

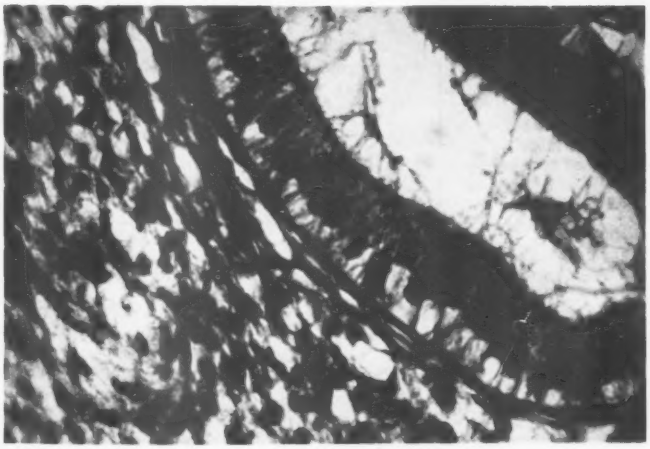

Fig. 3. Biopsy taken on the same patient: Secretory endometrium (2nd postovulatory day). (Dr. Peters).

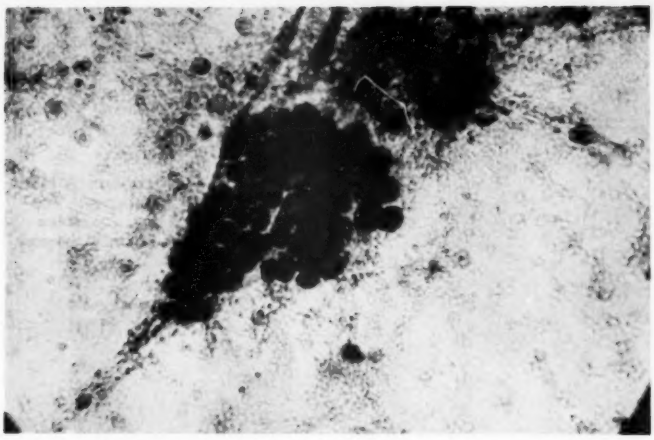

Fig. 4. Columnar cells in the late secretory phase show two types of nuclear degeneration: the small dark "burnt out" nuclei and the clear, structureless "shell-like" nuclei. (Dr. Peters).

Since not only the structures of the endometrium but also that of the individual cells shows a natural variation, the diagnosis from intra-uterine smears during the phases of the cycle is unreliable in many instances. We agree with Boschann that intra-uterine smears offer no advantage over the usually employed methods (diagnostic curettage, vaginal cytology, basal temperature determination).

MANUEL GALBIS, Valencia, Spain:

The histochemical features of the exfoliated cells obtained from secretory endometrium show the same irregular results as the proliferative ones. These wide variations are observed in relation to glycogen and pyroninophilic stain. The distribution of stain is very irregular and does not follow such a definite pattern as the one seen in endometrial tissue. We have been unable to establish a clear-cut difference between proliferative and secretory phase, except for an increase of glycogen in the latter and the pyroninophilic stain in the former; however, the variations are wide.

We do observe the presence of extracellular glycogen and pyroninophilic material in the secretory phase that could not be demonstrated in the proliferative phase. The nuclei in the secretory phase are round or oval shaped, somewhat clearer and show smaller nucleoli with pyroninophilic stain occasionally seen in the center.

NO CLOSING REMARKS

ENDOMETRIAL CYTOLOGY DURING AND AFTER THE MENOPAUSE

HANNS-WERNER BOSCHANN
West-Berlin, Germany

The following results were obtained on 128 patients (ages up to 75 years) with endometrial smears which were prepared either by brush or aspiration technique.

In the first years after menopause one often encounters the pattern observed during the proliferative phase of the menstrual cycle. In these cases one has either obtained cells from the functionalis of the endometrium which was still under the influence of follicular hormone in the absence of corpus luteum hormone stimulation, or (what is especially the case in uterine bleedings in menopause) one has obtained cellular elements from the basalis which are similar to the cells of the proliferative phase: dark nuclear staining due to marked chromatin content, oval to spindle-shaped nuclear form.

Sometimes one also finds the pattern of glandular cystic hyperplàsia: hyperchromatic, slightly anisokaryotic cells occasionally with an elongated cell form. Contrary to carcinoma, the chromatin pattern is regular and does not contain coarse clumps.

During senium usually all cellular elements exhibit a rather uniform pattern; the surface layer represents a low cylindrical cellular line. Glandular and stroma cells are smaller than during the reproductive years. The entire cellular pattern exhibits a so-called shift to the left (see paper entitled "Cytometry of Normal and Abnormal Endometrial Cells" in this edition). The nuclei are round to oval and often hypochromatic. However, hyperchromatic nuclei are also found. Then one usually deals with cells from the basalis. The evident isokaryosis of the senile endometrium makes the cytological differential diagnosis easy in uterine bleedings during menopause.

Histology	Cytology
Senile endometrium	Isokaryosis
Glandular hyperplasia, corpus polyp	Moderate anisokaryosis, but relatively finely granulated chromatin
Carcinoma	Marked anisokaryosis, marked hyperchromatosis, irregular distribution of the partly clumped chromatin
Sarcoma	Atypical stroma nuclei

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DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Münich, Germany:

The differential diagnostic scheme as suggested by Boschann appears very valuable for the recording of findings. In the senium the even shape of the small cells, usually crowded together with their nuclei, either hyper- or hypochromatic, is indeed very characteristic. It is hardly possible to mistake this for other functional conditions. Difficulty may be encountered in differentiating between malignant cells and those desquamated from a glandular-cystic hyperplastic endometrium or from an endometrial polyp in the menopause, especially when these cells are collected in the vagina after expulsion from the uterus and have already undergone degeneration.

JEAN A. DE BRUX, Paris, France:

I am in complete agreement with the excellent description by Boschann of the cytological characteristics of the endometrium in the different phases of the menopause. The typical cyto-pathological correlations are very clearly set forth. Unfortunately, in practice the problem is much more complicated than Boschann's paper would lead one to believe. The difficult problem of endometritis and of senile pyometria was not dealt with; and this, in my opinion, is the most knotty point, the one on which the largest number of errors have been made up to the present.

In these cases, the cells of the basal zone of the endometrium are profoundly modified. In the simplest cases they are reduced to a slender ring of cytoplasm, but the nucleus is very large, sometimes hypochromatic and sometimes, on the contrary, very chromatic and rather irregular. In the more complex cases one finds small exfoliated bands whose elements present a certain degree of anisocytosis and and anisokaryosis, and sometimes mitoses. Finally, the most difficult cases show nuclear debris of various sizes. However, all these elements are practically drowned in blood, modified polymorphonuclear leukocytes, lipophages and histiocytes. Thus, before applying the test of an anti-inflammatory and estrogenic treatment, it is impossible to state with certainty whether or not the lesion is benign.

GEORGE H. ROMBERG, White Plains, New York, U.S.A.:

I would like to ask Boschann if he has noted any cyclic variations in the endometrial pattern in some cases of the post-menopausal group. My own experience with such a group (age up to 73 years), where intermittent endometrial aspiration smears had been performed over a period of one to three years, showed that about 5% have such cyclic variations with normal-appearing endometrial cells occuring at intervals of three to five months. Although these "normal-looking" endometrial cells showed bright staining eosinophilic cytoplasm, no perinuclear vacuoles were noted.

CLOSING REMARKS

HANNS-WERNER BOSCHANN:

I would like to thank the discussants for their valuable completion of my own findings. I agree with de Brux, and Bickenbach and Soost, but I have not yet had the opportunity to follow up postmenopausal patients for a period of years as Romberg has done. Romberg's remarks are very interesting since similar spontaneous variations can be found in the vaginal smear.

CYTOLOGY OF ENDOMETRIAL HYPERPLASIA

ARTURO ANGEL ARRIGHI
Buenos Aires, Argentina

In conditions such as endometrial hyerplasia, many different patterns are seen in sections. We may find all the stages between the simple and clearly benign hyperplasia and the carcinoid-like hyperplasia (the so-called "unquiet" epithelium).

In smears obtained by endometrial aspiration the same situation occurs: smears from different patients, or even from the same patient on different days, show either normal cells or among the normal cells other types of cells that show abnormal characteristics. Anisokaryosis and irregularity in shape are among the most common nuclear alterations. In some cases these alterations are so remarkable that they give the cytological impression of malignancy.

When this type of abnormal cell is present in smears, a peculiar arrangement of the cellular elements is observed. Groups and clusters of cells are frequently seen, most of them deeply stained and having tightly arranged nuclei and very scant cytoplasm. They seem to be cells of stromal origin.

Some cells, possibly of endometrial origin (superficial or glandular), show more elongated and pale nuclei with more abundant cytoplasm.

It is our opinion that we cannot speak of definite patterns of endometrial cytology in endometrial hyperplasia. Different types of cells and cellular clusters can be seen according to the case. It is our feeling that endometrial hyperplasia can be suspected by endometrial or vaginal smears, but the general pattern of the smears has not been (according to our experience) clear enough to be sure of a correct diagnosis of endometrial hyperplasia. Nevertheless, in the presence of abnormal but non-malignant endometrial cells (described above) and vaginal cytology showing a high estrogenic level in a patient who complains of bleeding, endometrial hyperplasia can be reported with some degree of accuracy.

JEAN A. de BRUX and JACQUELINE FROMENT-DUPRÉ
Paris, France

Hyperplasia of the endometrium is a dystrophy characterized by exaggeration of the normal morphogenesis of the mucosa due to high and, more important, unbalanced hormonal stimuli. However, this state is reversible. It becomes permanent only after tissue reorganization and a lasting modification of its vascularization, with resulting changes in its histological aspect and finally in its cytological expression.

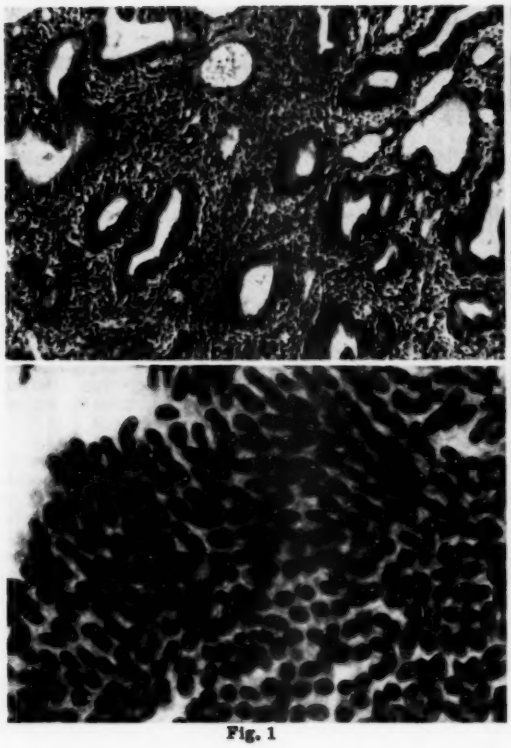

Fig. 1

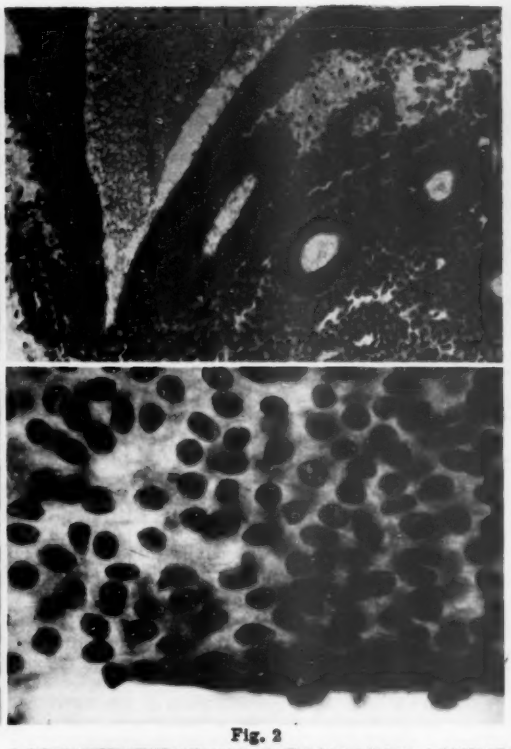

Fig. 2

Endometrial hyperplasia is a simple thickening of the mucosa due to an increase in the number and length of the tubes, the cellular coating of which is hyperplastic; whereas the fibrillary chorion is rich in voluminous, often mitotic, fibroblastic and reticular cells (Figs. 1, 2).

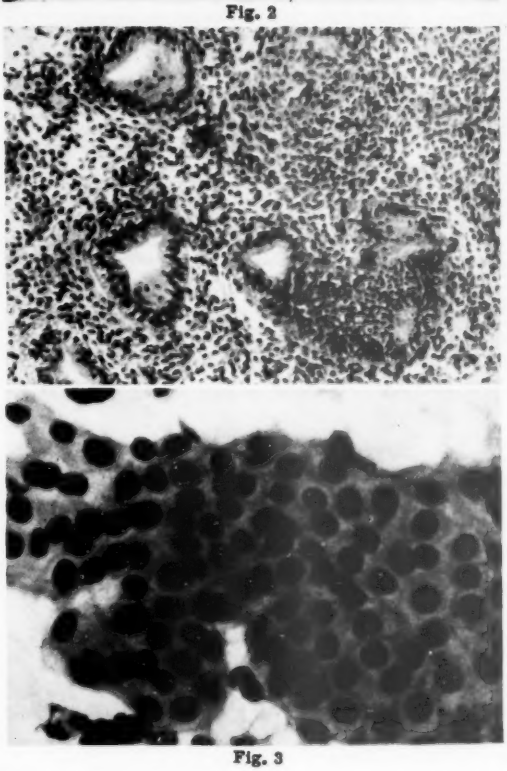

Fig. 3

Endometrial hyperplasia responds in an excessive and anarchic way to hormonal stimulation (Fig. 3). In this it differs from the "polyp," which no longer participates in the cyclic variations of the original mucosa.

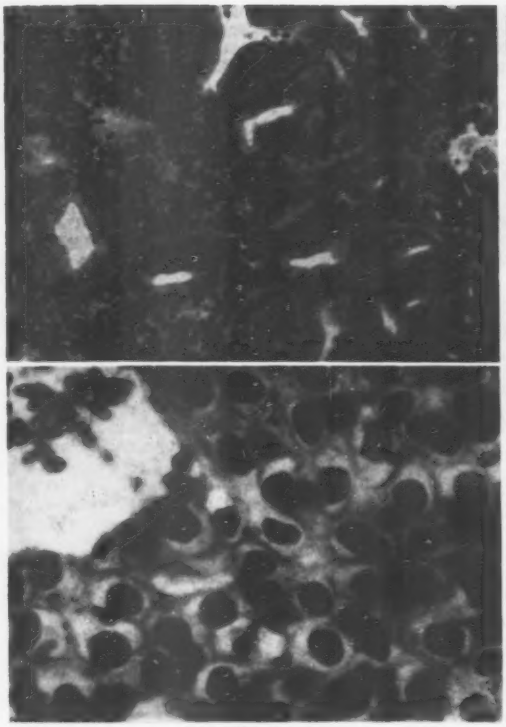

Fig. 4

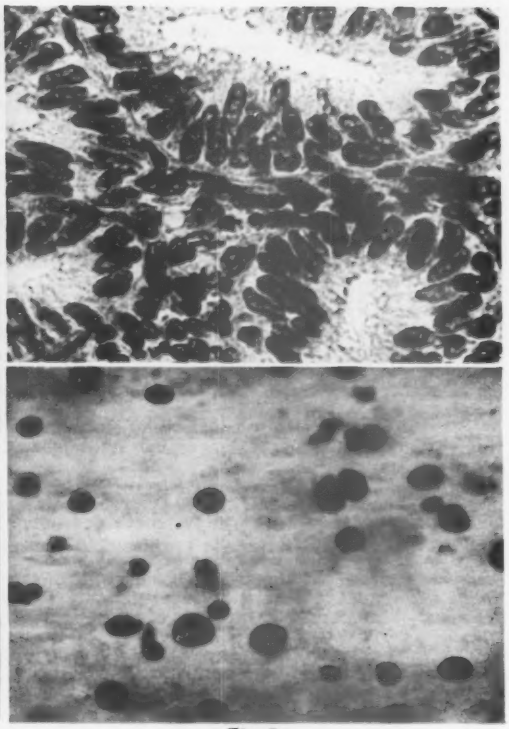

Fig. 5

In a certain number of cases the endometrial glands differ in size and are irregular in form (Fig. 4). Their border epithelium becomes hyperplastic and stratified (Fig. 5). Nuclear anomalies are found, characterized by increased volume, peripheral disposition of the chromatin and consequent vacuolar appearance of the central part. Mitoses vary in number and are generally normal, although anomalies may be noted. The cytoplasm appears swollen and at some points is eosinophilic (Fig. 6).

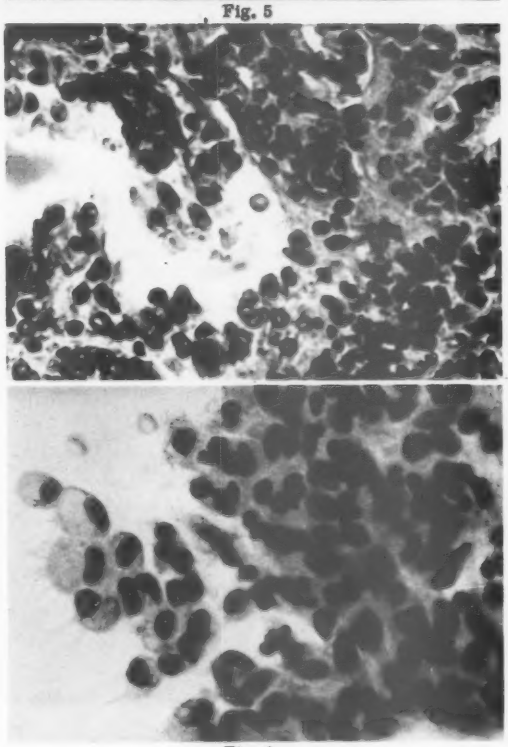

Fig. 6

These anomalies may be slight or marked, and in the latter case they raise the question of the ultimate cancerization of the lesion. In this connection Corcaden, Fertig and Gusberg have shown, based on extensive statistics, that women who present an endometrial hyperplasia have a 1.5% chance of developing a carcinoma. We have discussed the question of carcinoma in situ in another article in this edition.

Further, the endometrial hyperplasia which is surgically removed or spontaneously evacuated during a metrorrhagia or a menorrhagia recurs if the physio-pathological conditions are not modified. The ocytocics, which some are prone to inject as a means of enriching the smears, give little results here, unless the extremity is necrotic.

The exfoliated cellular elements of the hyperplasia, even if the architecture is abnormal, have a normal aspect, but the details appear more distinct, as if magnified. However, in some cases cellular alterations or degenerations raise diagnostic problems.

Morphology of the hyperplastic endometrial cell:

A description of a single cellular type would be an incomplete report of the diversity of the elements constituting the endometrium. Here, even more than in fragments exfoliated from a normal endometrium, it is sometimes possible to distinguish the cells of the cylindrical surface epithelium, of the glandular tubes and of the cytogenous chorion (endometrial stroma).

The hyperplastic endometrial cell, larger than the normal cell (15-20μ), shows a more extensive cytoplasm, polygonal or cylindrical in shape; it is basophilic or polychromatophilic and is usually vacuolized.

The nucleus is definitely central, round and clearly outlined. Its chromatin is very visible, forming a delicate network with two or three nucleoli strongly outlined.

The nuclear membrane generally shades in with the chromatin, but at some points it is distinctly outlined, whereas the chromatin appears to be formed of small granules in a discontinuous substance. These differences are essentially in relation to the different periods of the cellular function, as we have previously demonstrated by a study of the hepatic cell (1949).

These elements are most often grouped in fringes of distinctly cylindrical aspect. Very often one notes tissue fragments, having two aspects:

a) All the cells are piled together without precise cytoplasmic limits, giving an impression of round nuclei and having a characteristic relief like a heap of marbles (Figs. 1, 2); or
b) All the cytoplasmic limits are distinctly outlined, the nuclei are centrally placed and more flattened, and the ensemble resembles the scales of a serpent (Figs. 3, 4).

At the periphery of these cellular groups there appear naked nuclei, usually all of them identical, but sometimes with a slight anisonucleosis. Their chromatin forms a regular but denser network; sometimes one notes a peripheral crowding of the chromatin, leaving a sort of nuclear vacuole (Fig. 5).

A third variety of elements sometimes encountered consists of cells larger than those described above. The cytoplasm is more extensive, more basophilic and decidely round. The nucleus is very central and polymorphic (oval or elongated, sometimes kidney-shaped or notched). The chromatin is characteristically fine and regular. The cellular groups are always smaller and less compact than those of the other two varieties. Their arrangement appears looser and more disorderly, as if on the point of dissociating into isolated fragments (Figs. 6, 7).

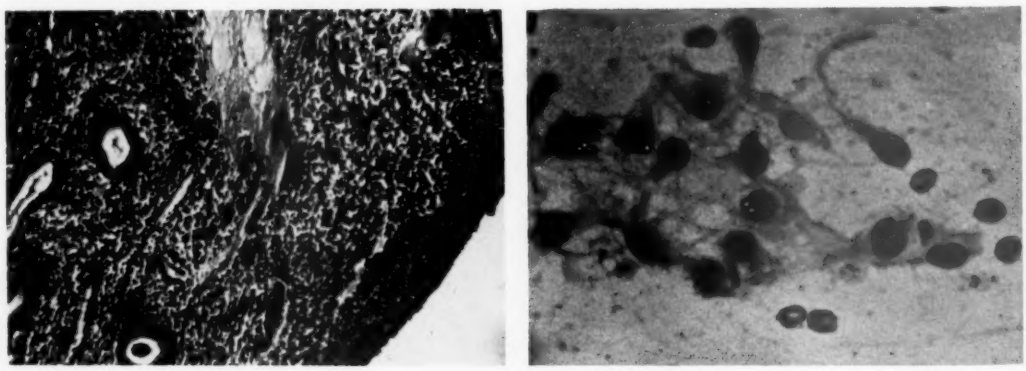

Fig. 7

We believe that this variety of cells corresponds to the elements of the stroma (cytogenous chorion), whereas the first two are probably exfoliated from the coating epithelium or from the glandular tubes. However this may be, this distinction would seem to require further precise histological correlations.

The cells undergoing degenerative changes (manifested by the presence of nuclear debris, swollen cylindrical cells with vacuolar cytoplasm and pyknotic or karyorrhexic nuclei) are relatively rare. They are the result of:

a) inflammation (rare in hyperplasia) or
b) very particular cellular modifications, such as postabortum endometrial hyperplasia with atypical cellular images as described by Arias-Stella.

Generally, the desquamation of isolated elements or of whole groups occurs in an unmixed fashion (in contrast to polyps).

Conclusion:

The diagnosis of endometrial hyperplasia can be made by the smear alone, for the smear associates:

a) direct signs: by the presence of desquamated hyperplastic endometiral elements in large numbers or in plaques;
b) indirect signs: the cytological context showing a sustained high estrogenic stimulation, or insufficient luteal signs;
c) negative signs: absence of abnormal elements and especially of associated inflammation (which is never encountered in cancer of the corpus uteri).

The other examinations, i.e., x-rays and the biopsy, should, of course, always be performed to confirm the cytological diagnosis.

CLARICE do AMARAL FERREIRA
Rio de Janeiro, Brazil

In cases of endometrial hyperplasia we have frequently seen isolated cells or cells in little linear groups, well differentiated, longer than the common proliferative cells, with basal nuclei (Fig. 1). In a few cases we have seen the round type of cells.

With the MacManus staining technique the cytoplasm appears rich, eosinophilic, with more being in the glandular cells than in the stromal ones.

Because of the predominance of a certain type of cell in each phase (early or late proliferative phase, early or late secretory phase), or the presence of proliferative or hyperplastic cells at the end of the cycle in non-ovulatory cycles, the endometrial cytology is an aid in the diagnosis of the hormonal stimulation of the endometrium. In many cases, if the endometrium is responsive, it is also useful in the diagnosis of ovulation.

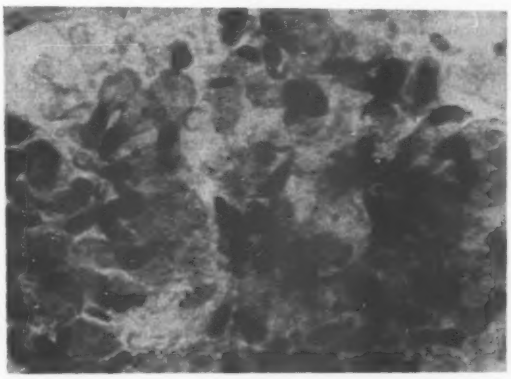

Fig. 1

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

The endometrial cytology of glandular-cystic hyperplasia often shows marked irregularity of the desquamated cell elements and their nuclei, which is exceeded only in cases of adenocarcinoma. We do not consider endometrial cytology of importance for the diagnosis of glandular cystic hyperplasia; on the other hand, it is very important in the diagnostic differentiation from malignant processes. The cancerous cell nuclei mostly have a more granular and irregular distribution of chromatin and manifest an even greater variation in size and shape. Often the nuclei in glandular-cystic hyperplasia are also denuded.

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

The variation of the cell picture in endometrial hyperplasia depends upon the actual hormonal situation of the hyperplasia. If there is a high estrogenic level, we see endometrial cells characteristic of the proliferative phase. Some of the signs of proliferation are exaggerated: more mitoses than usual, some degree of hyperchromasia, enlargement of some nuclei, long-tailed glandular cells and stromal cells with an enlarged cytoplasmic rim. Hyperplastic cells show a high variation rate, the square surface of the nuclei being: $51.1\mu^2 \pm 13.3\mu^2$. This variation in size allows a differential diagnosis in cases of postmenopausal bleedings: uniform small nuclei - senile endometrium; nuclei varying in size with regular chromatin pattern - hyperplasia; nuclear irregularities and highest degrees of variation in sizes - endometrial carcinoma. In the case of uterine polyps, cells may look similar to hyperplastic cells but usually do not show the same hyperchromasia. If there is a low estrogenic level (clinically: endometrium already shed but bleeding still going on) we only see an uncharacteristic smear picture, mostly cells from the basal layer without typical signs of function. In such cases the vaginal smear will be unreliable as well.

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MARCEL GAUDEFROY, Lille, France:

I would like to thank Arrighi for his very objective paper, and I quite agree with his excellent description. My feeling, like his, is that there are no definite cellular patterns of endometrial hyperplasia. In cases of intermenstrual or premenstrual bleeding, single, non-over-lapping endometrial cells are often found, and when the nuclei are more or less degenerate, it is almost impossible to say if these cells are glandular or stromal, malignant or non-malignant. These cells are larger, more irregular than the normal menstrual endometrial cells, and sometimes their variations in shape and size could be confused with malignant cells, if the chromatin of the nuclei were more deeply stained. But these atypical endometrial cells disappear at the same time as the bleeding, and aspiration during periods of non-bleeding shows that these fresh hyperplastic endometrial cells have a clear nucleus and not a dark and deeply stained one. Endometrial biopsy generally confirms the exact diagnosis.

I am much less optimistic than Clarice Ferreira on the hormonal interpretation of endometrial cytology. In my opinion the diagnosis of ovulatory or anovulatory cycles exclusively by endometrial cytology is very difficult. And also, the diagnostic accuracy is only of probability.

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

I would agree with Arrighi that it is difficult to speak of a definite cytologic pattern in endometrial hyperplasia. It is true that the cytologist may suspect hyperplasia if there are many superficial cells accompanied by active endometrial cells. Unfortunately, this cell picture does not occur often. It was present in only 17% of cases of hyperplasia. We have found it difficult to correlate the cytologic picture with the histologic diagnosis of hyperplasia. The great majority of the patients have a cellular picture composed almost entirely of intermediate cells - a perfectly normal finding for the postmenopausal patient.

In regard to the negative signs stressed by de Brux, I would agree with the absence of abnormal elements. It is true that the patient with carcinoma of the corpus usually does have inflammation, but not always. We have several cases with positive smears with few leukocytes, and there was no evidence of inflammation.

JULIETA C. de LAGUNA, Mexico, D. F., Mexico:

In vaginal and cervical aspirations the polymorphism of endometrial cells, related to different functional states or pathological conditions of the endometrium, is very clear. The authors present a comprehensive description of the various cellular characteristics of common observations. We agree on the feasibility of making the cytological diagnosis of hyperplasia of the endometrium. I would like to emphasize a few points:

(1) Nothing has been said concerning the importance of the correlation between the presence of endometrial cells and the phase of the cycle in which the smear is obtained. We consider the possibility of hyperplasia solely because of the finding of endometrial cells in a smear of a menopausal woman or in the intermenstrual period. Of course, this is not the only fact and there are other signs that point to the diagnosis: a cytological picture of high estrogenic value and the "tissue fragments," described so well by de Brux and Jacqueline Froment-Dupré.

(2) Occasionally, nuclear changes are minimal and there is a great uniformity in the cellular population as a whole. In this situation biopsies taken in some of our menopausal women have shown atrophic epithelia. We consider these to represent the atrophic part of endometria that simultaneously have zones of hypertrophy exfoliating cells which appear in the vaginal smear (the characteristics referred to above).

(3) More advanced hyperplasias have morphological pictures that make it difficult to differentiate them from cancers. Simple hyperplasia is diagnosed in our laboratory as Negative II; when the nuclear changes are more important, it is classified as Negative III and finally, when we are not too certain concerning its benign nature, Doubtful III.

YOSEUP S. SONG, Providence, Rhode Island, U.S.A.:

The characteristic features of hyperplastic endometrial cells seem to be well illustrated by the three speakers, and I confirm the morphological appearance of cells in cases of endometrial hyperplasia. However, I hesitate to support the absolute diagnostic accuracy of smear findings alone, in establishing the diagnosis of endometrial hyperplasia.

If the smear examined contained endometrial cells with the morphological appearance of hyperplasia which was described by the speakers, then a diagnostic curettage would be recommended.

GUILLERMO TERZANO and JOSÉ MARIA E. MEZZADRA, Buenos Aires, Argentina:

The description of endometrial cytology in endometrial hyperplasia, presented by Clarice Ferreira, Arrighi, de Brux and Jacqueline Froment-Dupré is very interesting. We agree with the authors that endometrial hyperplasia can be diagnosed by exfoliative cytology when the vaginal smears show high estrogenic level and there are clusters of endometrial cells. But when dealing with endometrial cytology there is always the peril of the false negative and the false positive reports. Hyperplasia of the endometrium is one of the "dangerous" lesions in cytodiagnosis, because any endometrium having hyperplasia may desquamate atypical cells in dense clusters (large cells, hyperchromatic nuclei, anisokaryosis, etc.).

We have studied the vaginal smears of 68 women having endometrial hyperplasia (39 out of the 68 also had an endometrial aspiration). Our reports have been: Class I (normal cytology), seven; Class II, forty-four; Class III (suspicious), sixteen. There was one false positive (Class IV) case.

CLOSING REMARKS

ARTURO ANGEL ARRIGHI:

I agree with the discussants in that there exist two cytological findings of importance in this pathological condition: clusters of endometrial cells or fragments of tissue, and vaginal smears showing marked estrogenic stimulation. Unfortunately, these findings are not constant, which makes the diagnosis often impossible and the interpretation of the smears only of limited value.

CLARICE do AMARAL FERREIRA:

Concerning some authors' comments on hyperplasia, I would like to point out one thing: It is quite different to study endometrial cells taken directly from the uterine cavity, especially from scrapping the endometrium, and endometrial cells taken in vaginal or cervical smears. The exfoliated endometrial cells in the vagina often show a different pattern. I think we cannot have confidence, for hormonal purposes, upon such cells. As Gaudefroy says, the endometrial cells found in cases of hyperplasia during bleeding (cells in the vaginal content) are degenerated, hyperchromatic and sometimes suspicious, and "these atypical endometrial cells disappear at the same time as the bleeding, and aspiration during periods of non-bleeding show that the fresh hyperplastic endometrial cells have a clear and not a darkly stained nucleus"; this agrees with our thoughts and findings.

Though we have not yet a solid basis to evaluate endometrial smears under the hormonal viewpoint, I think this subject has a potential interest and in the future, perhaps, we will depend more upon such findings.

THE DIAGNOSTIC ACCURACY OF HORMONAL EVALUATION BY MEANS OF ENDOMETRIAL CYTOLOGY

GEORGE H. ROMBERG
White Plains, New York, U.S.A.

Cytologic smear studies of the endometrium obtained by the endometrial aspiration technique utilizing the Papanicolaou stain have offered a new approach which is simple, painless and reliable in the evaluation of the morphologic changes in the endometrium.

Although aspiration studies in the proliferative phase of the menstrual cycle yield relatively less material than in the secretory phase, it is sufficient to permit a recognition of its functional state. The onset of the secretory activity in the glandular cells can be determined as early as two days prior to ovulation, as contrasted to the biopsy method which does not demonstrate this phenomenon until about two days after ovulation.

The special staining for glycogen content in the normal cycle yields essentially similar results in both cytologic and histologic studies of the same material on the same date.

In the normal cycle it is felt that the diagnostic accuracy of the cytohormonal evaluation compares favorably with that obtained by endometrial biopsy and in addition, offers new information in both the preovulatory and ovulatory phases regarding morphological data relating to the changes in the shape of the nucleus which has not previously been recognized.

In females of the reproductive age group showing abnormal cycles with disturbed proliferative or secretory phases, the diagnostic accuracy of hormonal evaluation by means of endometrial smears also parallels that obtained by biopsy. In evaluating atrophic and hyperplastic endometria during menopause the same fact holds true.

An inherent essential involved in any statement regarding the diagnostic accuracy of hormonal evaluation by endometrial smears is the competency of the technique involved; it must be such as to yield sufficient material (either exfoliative or abrasive) which is then fixed at once in order to avoid drying. It has been my experience that the use of the Papanicolaou stains gives superior results with regard to morphologic endometrial details.

The final evaluation of some of the interesting cytologic findings such as: (1) the bizarre appearance of the nuclei of glandular cells in some cases of amenorrhea following prolonged progesterone therapy, (2) the occasional appearance of supranuclear vacuoles in endometrial glandular cells in the mid-proliferative phase when supposedly only estrogen hormone is being elaborated, (3) foci of hyperplastic endometrial stromal cells in the proliferative phase in some cases of pelvic inflammatory disease, etc., must await further research.

Fig. 1

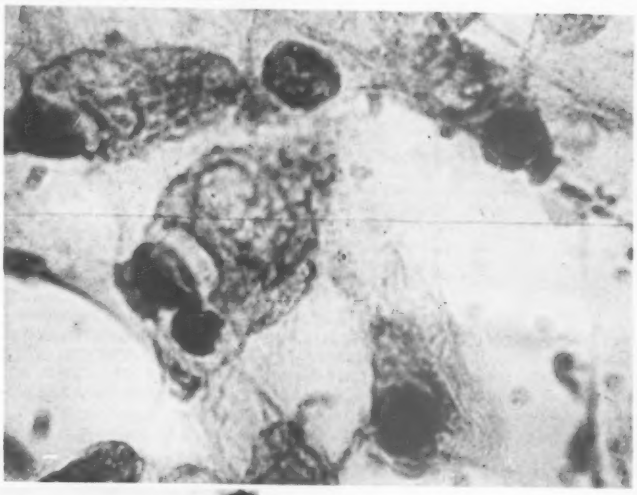

Fig. 2

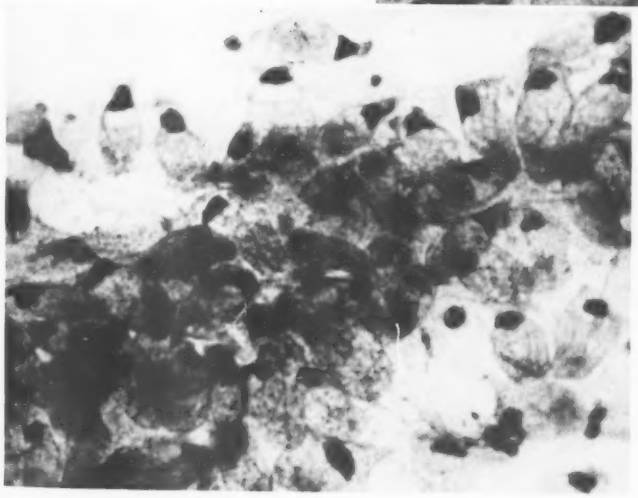

Fig. 3

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

Romberg's excellent studies on the hormonal evaluation of endometrial smears have shown that cyclic changes are not confined to the squamous vaginal cells. I would like to ask whether endometrial smears are able to give results concerning the hormonal situation of a patient which could not be given by the vaginal smears?

CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil:

As I have been studying the hormonal endometrial cytology based upon Romberg's work, I cannot but agree with his statements. Concerning the diagnosis of hormonal evaluations, it depends very much upon the cytologist's training. It is more difficult to interpret, in my opinion, the hormonal picture of the endometrium than of the vagina. Comparing the cytologic and histologic pictures in each case, through our experience, we feel that in the future we will modify the histologic conception of the cyclic endometrial changes. However, I think it is too early to try to prove its accuracy. But it is a fascinating study.

CLOSING REMARKS

GEORGE H. ROMBERG:

I wish to thank both discussants for their kind remarks. In answer to Boschann's question, I do feel that endometrial aspiration smears can give information concerning the hormonal situation which at times would be difficult or impossible by the vaginal smears. For example, in a current study of cases of amenorrhea, using various types of progesterone preparations, information obtained from endometrial smears was more useful in assessing the efficiency of the hormone therapy.

CORRELATIVE STUDIES ON ENDOMETRIAL CYTOLOGY, ENDOMETRIAL HISTOLOGY AND VAGINAL CYTOLOGY

WINIFRED LIU and RUTH M. GRAHAM
Buffalo, New York, U.S.A.

A correlative study was conducted using 374 patients to ascertain if a specific cytologic composition exists in the presence of a certain type of endometrium which may distinguish it from other types of endometrium. The endometrial tissues were available within 48 hours after the routine vaginal smears. They were obtained from both curettage and hysterectomy. Approximately half of the patients had a history of abnormal vaginal bleeding. No case of malignancy was included. Neither did these patients receive hormones before obtaining the specimens. About two-thirds of the patients were premenopausal. The histology of the endometrium was reported as being proliferative, secretory, hyperplastic or atrophic. The vaginal smears were counted for different forms of squamous epithelial cells: superficial, intermediate and basal. It seems that no remarkable correlative difference exists between curettage and hysterectomy materials. Neither did the history of menopause nor abnormal bleeding influence the results.

It was found that the superficial and the intermediate squamous cells in the vaginal smears bear little apparent correlation with the type of histology in the endometrium. However, in the 374 cases, only 58 had an appreciable number of basal cells (more than 10%) in their smears. Among them, 86% of the cases were reported to have an atrophic endometrium. In addition, among the patients who were reported to have proliferative, secretory and hyperplastic endometrium, 97%, 98% and 96% of the cases respectively, had only a minimum number of basal cells (less than 10%) in their smears.

It may be concluded that only the presence of basal cells in the vaginal smears is of significance in evaluating the type of endometrium. When the basal cells are present in a considerable number, an atrophic endometrium may be expected.

LUIS MONTALVO-RUIZ
Madrid, Spain

The systematic practice of endometrial biopsy in women with disturbances of the ovarian function, in the service of Prof. Botella and colpocytology done on the same day have permitted us to observe comparatively several hundred smears among the thousands that we have studied. We reviewed 300 smears of women who had had endometrial biopsy on the same day as cytology. We do not wish to comment on our personal results with endometrial cytology because as yet we have had little experience with it. Therefore, we will limit ourselves to a comparative study of endometrial histology and vaginal cytology. Our findings are reflected in the following table:

State of Endometrium	No. of Cases	Vaginal Smears			
		Estrogenic	Luteal	Atrophic	Infected
Proliferation	110	96	3	4	7
Hyperplasia	28	25	2	0	1
Secretion	134	4	119	4	7
Atrophy	28	3	2	23	0
Total	300				

Comments: In the 110 cases with proliferative endometrium we observed 14 smears not in agreement (correlation: 87.2%). But analyzing these cases we see that seven of them were infected smears, the majority by trichomonads, which disturbed the hormonal reading. One of the atrophic smears came from a woman who had intra-vaginal treatments with radium, and although she continued to menstruate, her vagina did not respond to ovarian hormones.

Of the 28 cases of hyperplasia, in two we obtained a luteal smear which corresponded to secretory hyperplasia and one infected smear. For these we can report perfect correlation.

In 134 cases with secretory endometrium we obtained agreement in 119, i.e., 89%. In seven an inflammatory process existed which could explain the lack of agreement. In the remaining eight we were unable to determine the cause of disagreement.

It is interesting to note that in a work which we published in 1954 we showed how the luteal cytology in its various phases; weak, normal and strong, corresponds to similar histological pictures of the endometrium, and in five cases with that history, luteal smears on the first five days of the cycle corresponded to secretory endometrium with persistence of the corpus luteum.

Because of this we, in accord with Guinot-Segui, Stoll and Jaeger, Allende and Orias, Roth, Pundel and others, estimate that vaginal smears permit an evaluation of luteal function which agrees with endometrial biopsy, with a negligible margin or error.

Of the 28 cases of atrophic endometrium, we observed in the vaginal smears three cases with estrogenic smears that corresponded, two of them from women with childlike uteri, one 23 years old with primary amenorrhea and well-developed sex organs except the uterus. The two had had large doses of estrogens, as demonstrated by serial cytology and estrogen excreted in the urine. We are unable to explain the disagreement with the biopsy of the remaining case. In another two cases of this series we observed two luteal smears from women with endometrial tuberculosis, so that actually in these cases it was a question of lack of receptivity of the uterine mucosa in two cases because of uterine hypoplasia and in the other two because of endometrial tuberculosis.

From the foregoing we can say in summary, that the correlation between vaginal cytology and endometrial histology is perfect, and that the percent of error is negligible.

PIERO SORA
Pavia, Italy

In a series of studies performed on twenty selected cases of hormonal disorders, chiefly of metrorrhagic type, the endometrium and the vagina have shown a different reactivity to the specific hormonal stimuli (8).

The disagreements between the results of histological examination of the endometrium and those of vaginal cytology, construed on the basis of the values of urinary steroid excretion determined at the same time (biologically active estrogens, total phenolsteroids, pregnanediol and 17-ketosteroids), have given us the opportunity of distinguishing the following groups of cases:

a - six cases with endometrial hyperplasia and a normotrophic colpocytological picture;

b - six cases with endometrial hyperplasia and a hypotrophic colpocytological picture;

c - three cases with histological features of hypotrophic endometrium extensively infiltrated by extravasated red blood corpuscles and vaginal pattern indicative of normal or deficient estrogen activity;

d - three cases showing variation in the qualitative type, because a hypertrophic endometrium with initial progestational changes is associated with a normotrophic vaginal smear without luteal cells;

e - two cases with colpocytological pattern indicative of a hyperestrogenic condition and histological picture of hypotrophic or atrophic endometrium.

As other authors (3,6,9) have maintained before us, a part of these cases must be referred to a different receptivity of uterine "effector" and/or respectively of vaginal "effector" to the sex steroids.

In some cases, although the reactive changes of the peripheral target-organs have shown something similar to what happens in allergic phenomena, they have been attributed to a condition of hypersensitivity of the endometrium or vagina to estrogens, because their metabolites were present in the 24-hour urine in normal or even subnormal quantities.

A morphologic documentation of the presence in the endometrium of a hyperreactive condition is given, according to the opinion of Guercio and Candiani (4), by the hypotrophic endometrium extensively infiltrated by extravasated blood, in which is evident the sharing of a very sudden vasomotor crisis with striking alterations of vascular wall permeability.

The vaginal hyperreactivity to subnormal estrogenic levels has been found only in two cases, in which it was not possible to evidence completely the presence of a hyperergic variation of vaginal epithelium reaction power. This is because the study of vaginal reaction by means of cytological examination does not allow us to point out the changes of vascular and connective tissue that are the only evidence of the hyperergic variation of tissue reaction.

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DISCUSSION

JEAN A. de BRUX, Paris, France:

The three papers submitted for discussion studied, with considerable statistical precision, the histology of the functional states of the endometrium and of the vaginal smear. However, it should be noted that none of the speakers discussed completely the question as it was stated: The question of the endometrial cytology in relation to vaginal cytology and endometrial histology was not even touched upon. Moreover, it was precisely because of the difficulty of this part of the question that I had seriously hesitated to take part in the debate, and had even considered adding no comments to those of the speakers.

Now to take up the subject as it was treated; I am almost in full agreement with the conclusions of Montalvo.

Since my personal experience at the present moment consists of several thousand endometrial biopsies and vaginal smears performed the same day, often with a study of the entire cycle, for the most diverse reasons (dysmenorrhea, amenorrhea, sterility), in women of all ages, I shall not include (a) the hyperplasias, the polyps and the malignant proliferations, and (b) the most frequent endometrial-vaginal correlations, and shall attempt to specify the discrepancies.

1. Low Karyopyknotic Index, with endometrium in proliferative phase. It is necessary to know how to interpret the actual state of the endometrium: it is usually thin, with few glandular tubes and rare mitoses. It may be supposed:

(a) that the cycle is long and that the follicular stimulation is slow to appear, owing to a "stagnation" of the ovum, as occurs in prepuberty or in premenopause, or following an abundant metrorrhagia; or

(b) that the quantity of folliculin secreted is sufficient to induce some mitoses, but still insufficient to produce an increase of all the mucosa itself.

At this point, one must have recourse to the clinical context: the case may be that of a young woman or young girl spontaneously amenorrheic, or that of a woman having given birth some months previously, and presenting no clinical signs of estrogenic deficiency. In such cases, a minimal quantity of estrogen will suffice to induce uterine bleeding at first anovulatory, later normal.

2. More or less elevated Karyopyknotic and Eosinophilic Indices, without or with few luteal signs, in middle secretory phase (21st to 24th day). In this case, a study of the endometrium shows either:

(a) a marked retardation in signs of luteal action; the endometrium resembles that of the 15th-16th day; or

(b) feeble signs of luteal action (vacuoles of glycogen at the base of the cells, but no spiral arterioles). There persist, however, signs of marked estrogenic action (pluristratification of the epithelial cells, and very numerous mitotic figures). This form is extremely frequent.

3. High Karyopyknotic and Eosinophilic Indices, the endometrium presenting the aspect of the premenstrual phase. The patient, moreover, often complains of "premenstrual syndrome." In this case we are concerned with the classic estrogenic thrust, but the endometrium is no longer capable of registering it; this is reflected only by the vaginal epithelium, like the female heart, a very sensitive and inconstant mirror.

4. The smear is composed of a mixture of superficial and intermediate cells, indicating marked hormonal stimulation; the endometrium is either quiescent or subatrophic. An attentive study of the cytological characteristics, and particularly of the excretion of the 17-Ketosteroids, will permit the crystallization of the cytological and histological patterns into the pattern of an "androgenia" (or, better, of an "adreno-androgenia").

Other examples might be cited of these so-called endometrio-vaginal discrepancies, or of the so-called differences in receptivity of the different organs. But such examples only reflect our inability to grasp the significance of the biological facts. Consequently, only a close agreement among the clinician, the endocrinologist, the pathologist and the cytologist will permit a correct interpretation of these facts.

MARCEL GAUDEFROY, Lille, Nord, France:

Along with Winifred Liu and Ruth Graham, Montalvo and Sora, I think there is a very good correlation between vaginal cytology and endometrial histology. I cannot compare these with endometrial cytology because my observations in this case are too few.

Intending to clear up the problem of the receptivity of uterus and vagina, I have compared 500 endometrial biopsies with about 3,500 vaginal smears in women with clinical hormonal troubles. For the most part, 3 or more smears were made in the same cycle as the endometrial biopsy, and always one smear was made on the day of the biopsy.

It was concluded that, in 35.2% of the cases, there was absolute agreement between both these ways of cytohormonal exploration. In 46.6% of the cases, there was a "complementary concordance." By this expression it is meant that in those endothelia which only show folliculinic action (proliferative, hyperplastic or atrophic state), as in those endometria which present a progesterone action (secretory phase), the study of vaginal smears affords more valuable and accurate information about the estrogenic impregnation (which is not clearly seen by biopsy alone). In 12.2% of the cases, there was an apparent disagreement, but this was easily explained by different clinical circumstances or by causes of error such as trichomonas infestation, vaginal douches and tablets, etc. In 6% of the cases, a real and unexplained disagreement was found. This observation emphasizes both the significance and the rarity of the local factors (Moricard's metrose de receptivity," i.e., receptivity-metrosis), as relatively easy to define through findings from two different modes of cytohormonal exploration.

To Sora, I must say that I have never observed hyperreactivity or non-receptivity of the vaginal epithelium, and I think these abnormalities are more frequently met in the endometrium.

WOLFGANG KORTE, Bonn, Germany:

By highly interesting studies Winifred Liu and Ruth Graham have established a correlation between endometrial histology and vaginal cytology in the atrophic menopause, i.e., during a time of complete estrogenic inactivity. From this it may be concluded that generally under fixed conditions, there may be a comparable relationship between different tissues in different parts of the organic system, which may have to be demonstrated by different methods.

There should be no comparison between the reaction of the endometrium and the vaginal epithelium to the same stimulus when one has a local pathologic condition and the other presents a normal appearance, as Montalvo has shown. Therefore, one should form a comparative study. By this investigation the conditions for a comparision of the histological endometrial picture with the cytological vaginal picture should be set.

It will be difficult to create presuppositions for such comparative studies in cases with no evident genital pathology, because there is no clinical indication for anything more than a smear. Sora has published significant studies on the conditions of vessels and vessel walls. A hypertrophic wall of the uterus, however, must have a better vascularization than a normal one. A better vascularization is accompanied with better conditions of circulation, which must act on the endometrium, too. Hyperemia of the uterus, however, does not mean hyperemia of the vaginal wall.

Finally, the time factor is of importance. We already pointed to the fact that different tissues have a different reaction time to the same hormonal stimuli. This is connected with the degree of differentiation of cells and tissues. One must consider, too, that the endometrium upon receiving the stimuli does not desquamate cells before menstruation. In the vaginal smear, however, cells are at hand, which are desquamated, and nevertheless, are compared to cells which are still in another tissue formation. From this it could be concluded that a histological picture of the endometrium, for example of the 14th day of the cycle, may correspond to a cytological pattern of the vaginal epithelium perhaps of the 11th or 12th day of the cycle.

Finally, the genital regions must be examined with regard to their sensitiveness to different kinds of estrogens. The threshold dosage of an estrogen varies for different tissues. An example can be shown in the so-called glandular-cystic hyperplasia of the mucous membrane of the endometrium. As is well known, this clinical picture, clearly defined by Robert Schroder and later on by Siebke, is able to show quite different forms which vary from hyperplastic structure to degenerative formation of cysts, to necrotic epithelial desquamation. The vaginal epithelium, however, is still in the stage of marked estrogenic proliferation, under the influence of this particular estrogen, which was not able to sustain the endometrium in proliferation.

Puck has pointed to the fact that estriol is able to effect a proliferation of the vaginal epithelium, whereas the quantity or the quality of this fraction of the follicular hormone is not yet able to effect comparable conditions on the endometrium. Consequently, it seems expedient to analyse all factors which visualize under suitable conditions, a correlation between the morphological picture of the endometrium and the vaginal epithelium, which certainly exists.

CLOSING REMARKS

RUTH M. GRAHAM:

I was interested in the 82% agreement between the cytologic and histologic material of Gaudefroy. I wish he had been more specific in describing the correlation. Since he includes proliferative, secretory and atrophic endometrium in one group, it is difficult to know what type of cell or cytologic picture he is referring to. Perhaps he could elaborate on this point in a letter to the editor. We did not regard our correlation as very good. We were not able to distinguish between secretory, hyperplastic or proliferative endometrium on the basis of differential counts. As a matter of fact, the only endometrium we were able to recognize with any degree of accuracy was the atrophic one.

Korte's point is well taken considering the time factor. It may well be that the time factors vary considerably between the vaginal epithelium and the endometrium and that the cells desquamated today received their stimuli many days previously. This might account for the lack of correlation.

De Brux has stressed the fact that there are discrepancies and that a satisfactory explanation can only be arrived at by the cooperative efforts of the clinician, the endocrinologist, the pathologist and cytologist. I agree with this completely. It seems that it is almost impossible from the cytologic picture alone to distinguish combinations of hormone stimulation. In our series, the only instance in which the cytologic picture could predict the state of the endometrium was in atrophy or in the absence of hormonal stimulation.

LUIS MONTALVO-RUIZ:

Indeed, there is very little basic descrepancy between the discussants and my work.

In answer to Dr. Gaudefroy: I think that 3 or more biopsies in the same cycle are excessive since a trauma occurs which can injure the patient. We do only 2 biopsies in the same cycle, at the very most, one in the first half and the other in the second half of the cycle. Naturally, it would be ideal to do daily biopsies and cytological smears during each cycle.

In answer to Dr. Korte: The fact that in certain cases damage might occur in either the endometrium or the vagina which might impede the reaction of either one, does not mean that correlations cannot be made in normal physiologic conditions, when the biopsy and smears are done on the same day and exhibit similar characteristics. So our conclusions are right. However, endometrial biopsies performed every day or every other day for the sole purpose of comparing cytology with histology does not seem feasible because one injures the endometrium with each biopsy. This injury does not heal within two or four or more days. So that the last biopsy from this scar tissue would not be of any value, not to speak of the fact that they are not necessary for our purposes.

In answer to Dr. de Brux: His thoughts concerning discrepancies between vaginal cytology and the endometrial status are very interesting, even though in many cases those discrepancies are apparent, since a complete clinical study will explain them correctly as he states in his point #4. In # 3, he speaks of cases with high Karyopyknotic and Eosinophilic Indices with an endometrium in the "premenstrual syndrome." In these cases, there exists hyperestrogenism demonstrated by cytology because the vagina is more responsive to hormones than the endometrium. In these cases we can speak of relative discrepancies but not absolute discrepancies.

I agree with him that only a close collaboration between the clinician, the endocrinologist, the pathologist and the cytologist will carry us to a correct interpretation of the facts.

PIERO SORA:

I would like to thank Gaudefroy, Korte and de Brux for their discussions.

To Gaudefroy and de Brux I would mention the importance of giving hormonal dosages before deciding if the disagreement between endometrium and vagina is caused by hypo- or hyper-reactivity of one of the two effectors.

The pathogenetic problem of the pathological hormonal reactivity of the vaginal epithelium has been discussed already by Champy and co-workers, by Toaff, by Cazzola and Sora and by myself (see bibliography of my paper).

I agree with Korte in regard to the "time factor" of these different tissues reaction, but I would reply that in hormonal disorders, especially characterized by menometrorrhagia, the reactional power of these tissues is frequently altered by the interference of local factors not yet completely known (endocellular enzymatic deficiencies, alterations of visceral neurovegetative system or others?). In my opinion, the true question to solve actually concerns this point.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

HORMONAL EVALUATION, BY MEANS OF VAGINAL CYTOLOGY, OF PATIENTS WITH ENDOMETRIAL CARCINOMA

GEORGE L. WIED
Chicago, Illinois, U.S.A.

It is the purpose of the present paper to describe the cytohormonal patterns in patients with endometrial adenocarcinoma and to compare these cytohormonal patterns with those observed in a comparative age group of healthy women. It is not the purpose of this presentation to enter into the discussion of whether or not steroid hormones are associated with the occurrence of endometrial adenocarcinoma. This is especially not feasible since the cytohormonal observations on the vaginal epithelium in patients with endometrial carcinoma cannot identify the actual hormonal condition at the time of the onset of the lesion, but only at a period some time after its onset. Only women without vaginal disturbances due to inflammatory reactions or infestations (other than atrophic vaginitis) were used in this study.

Cytological vaginal smears on 82 patients with adenocarcinoma of the uterine endometrium were evaluated as to their cytohormonal patterns. Of these 82 patients, 50 were more than 3 years postmenopausal, 20 were either less than 3 years postmenopausal, or exhibited apparent climacteric menorrhagias or menstrual irregularities, and 12 patients showed more or less normal menstrual uterine bleedings at the time when the vaginal smears were taken. The latter two groups, consisting of 32 patients with varying degrees of ovarian function could not very well be evaluated as to their cytohormonal patterns, since in many of them cyclic changes have to be considered which make the proper cytohormonal evaluation and comparison unreliable.

The cytohormonal patterns of the 50 (more than 3 years postmenopausal) patients with endometrial adenocarcinoma were compared with the cytohormonal patterns of 100 healthy women in comparable age groups and menstrual histories. In addition to these 1000 patients there was another comparative group of 100 patients who had undergone hysterectomy and bilateral oophorectomy more than 3 years previously.

The Karyopyknotic, Eosinophilic, Folded Cell and Crowded Cell Indices were evaluated in these 1150 smears. The following main cytohormonal patterns were distinguished: (1) atrophic type, (2) intermediate type (K.I. 0), (3) up to 5% superficial cells (K.I. up to 5), (4) more than 5% but less than 20% superficial cells (K.I. 5-20), and (5) more than 20% superficial cells (K.I. over 20).

The results of these classifications are shown in the following table:

	(1) Atrophic	(2) Intermediate K.I. 0	(3) K.I. Up to 5	(4) K.I. 5-20	(5) K.I. More than 20
50 postmenopausal patients with endometrial adenocarcinoma	38%	49%	12%	1%	
1000 healthy postmenopausal women in comparative age and menstrual history group	36.5%	50.8%	10.5%	1.7%	0.5%
100 castrates (hysterectomy and bilateral oophorectomy)	34%	61%	4%	1%	

From these results it can be stated that the postmenopausal patients with adenocarcinoma of the endometrium and the apparently healthy postmenopausal women of comparative age and menstrual history showed statistically insignificant differences in their cytohormonal patterns. Compared with the patients who had undergone hysterectomy and bilateral oophorectomy (more than 3 years before), it could be said that in this group there were more intermediate types (K.I. 0) and fewer cases in the next class of proliferation (K.I. up to 5).

Sixty-one per cent intermediate types (K.I. 0) and 34% atrophic types (together 95%) were found in those patients who had undergone both a hysterectomy and bilateral oophorectomy three years previously. This shows that the atrophic as well as the intermediate types may occur in the absence of any ovarian hormonal stimulation and any uncertain stimulant which might have its origin in the uterus. In other words, it does not seem justifiable to speak of an increased estrogenic effect when referring to the smear exhibiting an intermediate (K.I. 0%) type in castrated women as compared with those who exhibit the atrophic type, especially when there is no definite biochemical evidence to the contrary.

Similarly, this may hold true for the postmenopausal patients with ovaries, although the conditions are as clear-cut as in the group of surgical castrates. In any case, in our material from 50 (more than 3 years) postmenopausal patients with endometrial adenocarcinoma, there was no evidence that there is a significant statistical difference in their cytohormonal patterns as compared with a group of 1000 healthy postmenopausal women in the same age and menstrual history group. It should be emphasized that the vaginal smears of patients with disturbing vaginal inflammatory reactions, infestations or other disturbing factors (pessary, prolapse, irradiation) were not included in this study since these changes usually preclude proper hormonal evaluation by means of cytological smears.

In summarizing these findings, one may say that there was no evidence in our material that the postmenopausal patients with endometrial adenocarcinoma showed a significantly different cytohormonal pattern than the healthy postmenopausal women in the respective age groups. Which hormonal pattern existed at the time of onset of the lesion cannot be stated, but it can be shown that there was no statistically significant increase in estrogen stimulation present in the postmenopausal women at the time when endometrial adenocarcinoma was diagnosed, as evidenced by examination of vaginal smears.

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DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Münich, Germany:

Our observations agree fully with the results published by Wied. We did not see any signs of increased follicular hormonal activity in vaginal smears taken from patients with carcinoma of the uterine body at the time when the carcinoma became clinically obvious. If we do not find signs of marked follicular hormonal activity, in patients with bleeding after the menopause, we consider it all the more necessary that the specimen be examined with particular care for atypical cells. Curettage is strongly advised in these cases.

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

In our material of 96 patients with endometrial adenocarcinoma, we saw a 5-30 K.I. only in three cases. So we confirm Wied's statement that patients with adenocarcinoma show the same cytohormonal pattern as healthy postmenopausal women. The inflammation accompanying endometrial

carcinoma must be considered as an important source of error in this problem, since it oftens causes pseudo-eosinophilia and pseudo-karyopyknosis simulating an estrogenic effect. In such cases, local antibiotic therapy reveals the true cytohormonal pattern.

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

When I studied Wied's paper on "The Hormonal Evaluation by Means of Vaginal Cytology of Patients with Endometrial Carcinoma," I was surprised to see such a low Karyopyknotic Index in the carcinoma group. Previous work in this laboratory had indicated that the patients with endometrial cancer had more superficial cells than normal women of identical age.

Because of this discrepancy, I did counts on fifty consecutive patients with cancer of the corpus admitted to Roswell Park Memorial Institute since February, 1957. All of the patients were postmenopausal except one and in general they were an elderly group, thirty per cent being seventy years or older. Nine patients were in their fifties.

The cellular picture in these fifty cases varied considerably from that presented by Wied. In the first place, the picture of many intermediate cells and no superficial cells was present in only three cases. In the atrophic group, there was closer agreement - since 13, or 26%, of the cases had atrophic smears, which is little different than the 38% shown in the table by Wied.

When the Karyopyknotic Index is considered, there is really a great difference between this material and that of Wied's. Though he reports only 1% of the patients having a Karyopyknotic Index over 5, in our material 28% fall in this group. Wied has no patient in the carcinoma group with a Karyopyknotic Index over 20. Nine patients in our series had a Karyopyknotic Index over 30, or 18% of the cases.

Since this Institute is a cancer hospital and only patients with cancer or those suspected of having cancer are referred here, it is impossible for us to compare the cellular picture of patients with cancer of the fundus to normal controls as Wied has done. Instead, we have compared them to a group of 31 patients seen during the same period with postmenopausal bleeding but in whom no cancer was demonstrated. Twelve had hyperplasia of the endometrium; nine, atrophy of the endometrium and the remaining twelve had polyps, fibroids, etc. The cellular picture in this group was not statistically different from that of the carcinoma of the corpus group. Nineteen per cent were atrophic compared to 26% of the cancer group. Ten per cent had a Karyopyknotic Index over 30 as compared to 18% of the cancer groups. In such small numbers of cases this difference is not significant.

There is a real discrepancy between the cytohormonal patterns found by Wied and those found in our material. This raises several questions. Is it a difference in clinical material? If anything, the cases of Wied are younger than those in our series and so, relative age of patients can probably be excluded.

Is it a difference in interpretation? I, too, excluded patients with disturbing vaginal infestations. In the Symposium on Terminology, Wied and I agreed in every instance on the differentiation between intermediate and superficial cells, so one would assume we are classifying cells in a similar fashion. I would like to ask if these hormonal readings were done on stained preparations or by phase microscopy? This might account for the difference.

In summary, we find that there were a significant number of patients with carcinoma of the corpus with Karyopyknotic Indices over 30. Whether or not estrogen, per se, is implicated in the etiology of fundal carcinoma is impossible to say, since as Wied says, these are the cytohormonal patterns when the disease is established, not at the time of the beginning of the tumor. Twenty-eight per cent of the patients had atrophic smears, suggesting again the difficulty of correlating estrogen stimulation with the etiology of this lesion. The cytohormonal patterns of those patients with carcinoma of the fundus were not any different than those of postmenopausal patients with bleeding but no cancer.

PIERRE HAOUR, Lyon, France:

I approve of Wied insisting in his paper on the conditions to be met if one wants to compare the results in patients with endometrial carcinoma with results in healthy patients. First, it is necessary to consider the duration of menopause: Patients in the two groups should be chosen with similar menopausal durations, and at least 3 years after cessation of menstruation. This factor appears to me more important than the inflammatory and infestation reaction which in most cases does not affect the pyknosis (while the cytoplasmic appearance is evidently modified more by pessary and prolapse). However, I agree that it is safer to consider, if possible, only cases without possible disturbing factors. To discuss Wied's paper, we have been reviewing some of our results and have found it difficult to compare them with the large series of Wied and to select menopausal cases without any inflammation. Nevertheless, we studied 48 cases with adenocarcinoma and compared the vaginal formula with those of 200 postmenopausal healthy women. Half of this latter group had an urinary sediment smear instead of vaginal cytology, the cytological index being converted into the vaginal values applying the Lencioni formula (1). We found the following figures:

	Atrophic	Intermediate	up to 50% K.I.
200 healthy postmenopausal patients	16%	58%	26%
48 with endometrial carcinoma	10%	54%	35%

These percentages, especially when atrophic smears and smears with a Karyopyknotic Index up to 5% are considered, appear different from the values found by Wied and others (2). The percentage of atrophic cases is much lower in the two groups and there is a difference of 6% between the cancer and the normal group. It would seem, therefore, that in our experience there exists a cytological pattern which shows some differences in adenocarcinoma. But we are aware that our series of cases is not large enough to draw any definite conclusions.

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CONSTANTIN HEROVICI, Villejuif, Seine, France:

On the whole, we agree entirely with the very interesting presentation of Wied.

Nevertheless, we feel that we may be able to contribute something by submitting some of the ideas which have evolved from the cyto-histological study of a series of 18 cases of carcinoma of the uterine body.

Normally we classify the colpocytology of women who are postmenopausal into one of the following groups:

I. Atrophic or androgenic smears.
II. Mixed menopausal smears (i.e., smears that contain cells from all the various vaginal layers, and which have an Eosinophilic Index of 1 to 10, Karyopyknotic Index from 1 to 20, intermediate cells and basal cells). This appearance seems to reflect the persistence of a very slight estrogenic stimulation at the site where the vaginal smear was taken and where atrophy is taking place.
III. Vaginal smears that show moderate estrogenic stimulation with an Eosinophilic Index of from 1 to 10, and with intermediate cells but no basal cells; which is proof of the fairly high estrogenic stimulation, with the three vaginal layers developing even after the menopause.
IV. Hyper-estrogenic vaginal smears with an Eosinophilic Index of over 20, intermediate cells but no basal cells.

A series of 18 carcinomas of the uterine body have been investigated from the hormonal point of view and they have been divided as follows:

3 patients having atrophic or androgenic smears
9 patients having mixed menopausal smears
3 patients having estrogenic smears
3 patients having hyper-estrogenic smears

This distribution, when related to the 2000 vaginal smears of menopausal women that have been studied at the Institut Gustave Roussy since January, 1957, does not enable one to draw any conclusions as to the appreciable differences that might be characteristic, from the hormonal point of view, of endometrial cancers.

If, on the other hand, one divides the 18 cases of carcinoma of the uterine body according to the age of the patient, one obtains the following distribution:

1. Patients between 50 and 60 years of age
   3 atrophic smears
   2 mixed smears
   2 estrogenic smears
   3 hyper-estrogenic smears

2. Patients between 60 and 70 years of age
   1 estrogenic smear
   7 mixed smears

Although this series contains very few cases, one has the impression that the longer postmenopausal, the closer the hormonal aspect of the uterine body resembles that of cancer. This is nearly always due to the persistence of estrogenic stimulation for a long time after the menstrual periods have ended.

Moreover, from the histological point of view, we find in the first group, two purely columnar epitheliomas and 8 mixed squamous and columnar epitheliomas, while in the group of older women, we find 7 purely columnar epitheliomas and only one mixed epithelioma.

This seems to single out at least two types of cancer of the uterine body. One appears fairly soon after the menopause and originates either from a squamous cell metaplasia of the endocervix or endometrium or from an extended growth of a cervical carcinoma. The other, appearing much later after the menopause, is characterized by the persistence of estrogenic stimulation, which manifests itself histologically in the malignant transformation of a glandular hyperplasia of the endometrium.

CARLO MOSETTI, Torino, Italy:

In our research in 48 cases of endometrial carcinoma, although vaginal patterns of estrogen stimulation were present in excess in a small number of patients, they did not reach the particularly low values that Wied reported. In fact, such a cytohormonal pattern was found in 14.2% of the cases.

With reference to the main speaker's observations that no difference would exist between the hormonal conditions of women with endometrial carcinoma and that of healthy women of the same age, we think that it is difficult to characterize the hormonal conditions of women with endometrial carcinoma, for it is possible that there are alterations in the receptivity of the different genital sections.

The presence of anomalies in the sensitivity of these tissues has been demonstrated in our research by the histological test of the endometrium surrounding the carcinoma, which has shown patterns of glandular cystic hyperplasia in 23.8% of the cases (this condition is commonly thought to be an expression of estrogenic stimulation).

Consequently, there exists a significant statistical difference between vaginal and endometrial findings, in opposition to that which is observed in patients of the same age affected with a simple endometrial hyperplasia, in whom there has been found a concordance between the cytohormonal and endometrial patterns in more than 80% of the cases (1).

Therefore, we think that the cytohormonal test is not always sufficient to evaluate the hormonal conditions of women with endometrial carcinoma, so that other diagnostic tests are necessary, particularly those concerning the conditions of the endometrium.

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GUILLERMO TERZANO, Buenos Aires, Argentina:

It is indeed gratifying to read Wied's paper. We have been looking (hormonally speaking) for abnormal vaginal cytology (abnormal Karyopyknotic and/or Eosinophilic Indices) in the smears of postmenopausal women with endometrial carcinoma. In our series of 76 cases, the cytological picture was just as expected. We did not find the Karyopyknotic or the Eosinophilic Indices significantly higher (as described) than they should be for the age of the patient, years after menopause, etc.

We cannot discuss a paper we completely agree with.

ERICA WACHTEL, London, England, U.K.:

I am very surprised to see that Wied's estrogen assays in women suffering from endometrial cancer are so completely different from my own findings, although there is conformity of results in healthy postmenopausal and postcastration patients. My findings are tabulated below:

	K.I. high (over 10%)	K.I. low (under 10%)
40 postmenopausal adenocancer patients	33 (82.5%)	7 (17.5%)
200 healthy postmenopausal women	5 (2.5%)	195 (97.5%)

The explanation for this discrepancy may be found in the different methods of estrogen assesment. Wied records the Karyopyknotic Index as the percentage of cells with pyknotic nuclei from the total cell population, whereas we estimate the Cornification Index as the percentage of pyknotic cells among the superficial and intermediate squames only, that is, we exclude the basal cells from the K.I. estimation. We believe that inclusion of basal cells into the count confuses the picture, since deep cells are not only present in atrophic conditions due to estrogen deficiency but also in inflammatory conditions due to loss of surface epithelial layers. Carcinoma patients nearly always harbor concomitant inflammatory disease and, therefore, we consider the percentage of basal cells a misleading gauge of

hormonal status. For the same reason we disregard the Eosinophilic Index, which is unreliable in the presence of infection.

According to our findings, using the technique described above for estrogen assay, there seems to be a significant elevation of the Karyopyknotic Index in postmenopausal patients suffering from carcinoma of the endometrium.

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CLOSING REMARKS

GEORGE L. WIED:

The rather divergent observations of the discussants, who have contributed valuable data and viewpoints, show that it would be useful to pool the material and to discuss the issue again, after we have agreed on the standardization of some terms and indices.

Since Bickenbach and Soost, Boschann, Herovici and also Terzano expressed themselves as agreeing with our data, there remains only for me to thank them for their contributions.

Ruth Graham and Erica Wachtel who reported considerably higher average Karyopyknotic Indices on their material (together 90 cases) will agree with me that we all individually have too few cases on hand to give significant figures which could be expressed in percentages. Boschann with his 96 cases found Karyopyknotic Indices of above 5 only in 3 cases.

The figures by Haour are difficult to comment upon since his table does not show a breakdown in into comparable groups as in our table. However, although his figures are different from ours, his material shows the same statistical insignificance as ours: his 48 cases with endometrial adenocarcinoma show statistically no definite significance in comparison with his group of healthy women in similar age groups.

The extensive contribution by Herovici indicates to me that we need standardization of our terminology. Terminologies of "very slight estrogenic," "moderate estrogenic" and "hyper-estrogenic" seem to me to be too subjective.

As far as the suggestion by Mosetti is concerned, (to always add endometrial histological evaluations of the hormonal condition,) I believe that this has been done excessively, especially by those authors who wished to prove or disprove the fact that endometrial carcinomas coincide with glandular hyperplasias in statistically higher percentages than with patients without adenocarcinoma, in comparable age groups. It also remains to be considered that the endometrium often shows different patterns than would be expected from the vaginal epithelial patterns, since the response of the endometrium is in some respects less specific and less rapid, and it shows a sum of accumulated estrogenic and/or progestational stimuli which might have already lost their stimulative influence on the vaginal epithelium some time ago.

The figures of Erica Wachtel are again very difficult for me to discuss, since she separated her material into groups differently than I did in my paper. However, I would like to make a minor correction in her statement where she explains how I record the Karyopyknotic Index. My Karyopyknotic Index is identical with the Superficial Cell Index. I understand a superficial cell to be a large squamous cell with squared-off sharp edges containing a completely pyknotic nucleus.

The question of Ruth Graham concerning whether or not our hormonal evaluations were made with the phase microscope touches an important point. Yes, we made all of our counts of the Karyopyknotic Indices on stained material under the phase microscope. Only those which exhibited the peculiar red light effect (Fertility and Sterility 6: 61-65, 1955) were determined as "pyknotic." One can very well imagine that one might have more cells classified as containing a pyknotic nucleus if one uses a regular bright field microscope.

In order to shed more light on this rather interesting question, I would propose to the discussants, and to the readers, that we pool our material using the following patients, standards and criteria:

(a) Patients: only patients five years (or more) postmenopausal at the time of the detection of the lesion.
(b) Condition of Smear: all cases to be excluded from evaluation which show trichomonas infection and other disturbing vaginal infections. Patients with senile atrophic vaginitis may be included, however. Furthermore, patients are to be excluded with treated or untreated descensus uteri or prolapse and those who have had gynecological radiation therapy of any dosage at any time.
(c) Classification of Findings: the cytological findings shall be classified into the following groups:

1. atrophic or predominantly atrophic
2. intermediate or predominantly intermediate, but no superficial cells
3. 1-4% superficial cells (Karyopyknotic Index 1-4)
4. 5-9% superficial cells (Karyopyknotic Index 5-9)

5. 10-19% superficial cells (Karyopyknotic Index 10-19)
6. 20-29% superficial cells (Karyopyknotic Index 20-29)
7. 30-49% superficial cells (Karyopyknotic Index 30-49)
8. 50% and more superficial cells (Karyopyknotic Index of more than 49)

(d) Criterion of Karyopyknosis: fixed and stained nucleus smaller than 6μ or fixed and stained nucleus which is dense enough to cause an aberration (red) of the light under the phase microscope (Zeiss-Winkel Phasemicroscope 40x magnification). See pp. 38-40 and 48-53 in Vol. II, No. 1 of Acta Cytologica.

If the above discussants and the readers would agree to evaluate their material according to these terms and to send their data to the editorial office, it could be expected that one would be in a better position to make a statement as to the hormonal condition at the time of detection of the lesion.

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ABSTRACTS

This portion of ACTA CYTOLOGICA includes abstracts (approximately 150–300 word each) of papers, either recently published or accepted for publication. Authors are invited to submit their own abstracts. Authors are requested to forward to the Editorial Office a complete manuscript or reprint of the original paper together with their abstract. All figures should be included.

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CANCER CYTOLOGY

VALUTAZIONE STATISTICA DEL METODO CITO-DIAGNOSTICO NELLA PROFILASSI DEL CANCRO DEL COLLO UTERINE. L'ASPETTO CITOLOGICO DELLE PSEUDOEROSIONI PAPILLOMATOSE. (STATISTICAL EVALUATION OF THE CYTOLOGICAL TECHNIQUE IN THE PROPHYLAXIX OF CERVICAL CARCINOMA, WITH SPECIAL CONSIDERATION OF THE PAPILLOMATOUS PSEUDO-EROSIONS)

PAOLO ROMEO - Revista Italiana de Ginecologia 44: 319-328, 1958

After a brief review of the literature, the author reports the results of a four year study in the Department of Obstetrics and Gynecology of the University of Pisa. The study concerned the prophylaxis of cancer of the uterus and particularly of the cervical carcinoma. The author examined 1155 patients with cervical erosions by means of the cytological, surface biopsy method. The method has been fully satisfactory. 19 cases of cancer of the uterine cervix were revealed by it. There were 43 false positive reports and no false negative diagnoses.

The causes of error were erosion in prolapsed uterus, cervicovaginitis from Trichomonas vaginalis and chronic cervicitis. Papillomatous pseudo-erosions often give cytologic patterns which are difficult to evaluate. The presence of a papilla in the smear is pathognomonic of such of type of erosion. (Author's abstract.)

COMMUNITY SURVEY FOR UTERINE CANCER

KIMMELSTIEL, P., BOS, J.F., and NOLEN, C. - Obstetrics and Gynecology 11: 688-695, 1958

1. A stastical analysis of a self-sustained community cytology survey through private physicians' offices based on genital cell studies of 48,697 women has been presented.

Of the white female population, 21 years and over, 30.4 per cent were examined in 1 year.

The prevalence of uterine cancer in this series was 0.89 per cent. That of clinically unsuspected carcinomas was 0.56 per cent.

Of the total number of carcinomas of the cervix, 67.1 per cent were in situ, 28.2 per cent were infiltrating carcinomas.

The peak prevalence of infiltrating carcinomas was between the ages of 51 and 60, that of carcinoma in situ, between the age of 31 and 50, that of marked epithelial atypicalities, between the ages of 21 and 30.

2. The accuracy of cytologic interpretation has been discussed in terms of false positive and false negative reports.

3. For assessment of statistical evaluation, the importance of equivocal findings in cytologic and tissue examinations has been stressed.

4. Factors making a comparison of available statistics difficult have been discussed and a table of standardization has been suggested. (Authors' summary.)

DIE ZYTOLOGIE IM RAHMEN DER KREBSDIAGNOSTIK AN DER I. UNIV.-FRAUENKLINIK WIEN (CYTOLOGY AS A CANCER DETECTION METHOD AT THE 1st UNIVERSITY CLINIC FOR OB. AND GYNE., VIENNA)

R. KLEIN, E. KOFLER, H. KREMER and G. RHOMBERG - Geburtshilfe und Frauenheilkunde 18: 264-270, 1958

As a routine procedure at the 1st Department of Obstetrics and Gynecology, University of Vienna, Austria, during the time 1954-1956, 10,688 patients were examined cytologically in addition to Hinselmann's colposcopy and Antione-Grunberger's colpomicroscopy. The accuracy of cytology in the detection of carcinoma of the uterine cervix rose from 85% to 94% during the last six months, while the false positive results decreased from 113 patients (2.8%) to 16 patients (0.47%) in the last series of 3,377 patients. The importance of the application of all three early cancer detection methods is pointed out, since, when applying just one method only, an uncertain number of cases falsely grouped as negative is not evident. So-called statistically proved differences in efficiency of the various early cancer detection methods should not induce the abandoning of any of them as long as each shows certain sources of error. Applying Papanicolaou's classification also to colposcopy and colpomicroscopy is suggested. (Authors' abstract.)

HISTOLOGIE UND CYTOLOGIE IN DER GYNÄKOLOGISCHEN FUNKTIONSDIAGNOSTIK: 1132 FÄLLE (HISTOLOGY AND CYTOLOGY IN FUNCTIONAL DIAGNOSIS: STATISTICAL EVALUATION OF 1132 CASES)

PETER STOLL and OTTO LEDERMAIR - Gynaecologia (Basel) 145: 270-278, 1958

In 1132 patients the cytohormonal readings are compared with the functional findings in curettage. Absolute conformity was found in 78.5% of the cases, while in 17% conformity was found possible after closer evaluation. Differences are mostly explainable on the basis of smaller threshold values and quicker response of vaginal epithelium to ovarian hormones. The findings are subdivided into: proliferative stage, secretory stage, glandular hyperplasia, abortion, menopause and menstruation. The critical use of both examinations combined with anamnesis and palpation findings provide the best method for functional determination of ovarian activity and give valuable information for special hormonal treatment. This is especially true in cases of hyperplasia of the endometrium following hyper-estrenism. These cases can be divided into 5 groups: follicular persistency, follicular persistency with decrease in hormonal production, follicular atresia, corpus luteum development after follicular persistency with insufficient secretion of progesterone and corpus luteum development with small progesterone secretion. (Authors' abstract.)

THE CYTOLOGY OF CANCER OF THE UTERINE CERVIX

PETER STOLL - Acta Union Internat. Contra Cancrum 14: 314-320, 1958

Vaginal cytology is a valuable tool for the study of ovarian activity and the local epithelial atypias (inflammatory changes, metaplasia, carcinoma). If carcinoma is present, histology has to make the final diagnosis. Cellular morphology and histochemistry are used to study the differentiation of the epithelial cells:

Normal differentiation: normal cytological findings with typical nuclear structure and cytological maturation with loss of mitotic activity (congruence of maturation within cytoplasm and karyoplasm).

Abnormal differentiation: abnormal cytological figures, atypical nuclear and cytoplasmic maturation with persistency of mitotic activity (incongruence of maturation of cytoplasm and karyoplasm).

Usual methods (phase contrast microscopy, Papanicolaou staining, Cusmano reaction) are sufficient to make routine diagnosis and to make a certain record of suspicious and non-suspicious findings. (Author's abstract.)

CERVICAL EROSION AND ITS SIGNIFICANCE IN THE EARLY DIAGNOSIS OF CARCINOMA OF THE CERVIX

PETER STOLL, H. G. BACH and M. OPPITZ- Deutsche Medizinische Wochenschrift 83: 146-149, 1958

Of 16,749 out-patients examined in 1951-54, 3,299 had a "red-spot" on the cervix. Among these, 345 cases of cancer of the cervix were diagnosed with the naked eye. Another 56 cases of carcinoma of the cervix were discovered through colposcopy and cytology within the area of the "red spot." Among the

remaining 13,450 patients with macroscopically smooth cervices, employment of the special diagnostic methods, colposcopy and cytology, led to the discovery of a further 10 cases of cervical carcinoma. (Preinvasive processes have not been included.) The frequency of carcinoma of the cervix is thus 1 in 53; whereas with a macroscopically smooth and normal cervix it is 1 in 1,345.

The immediate aim of early diagnosis of cancer is the complete investigation of every "red spot" on the cervix. The use of special diagnostic methods in all patients over 35 is the ultimate aim. Erosions should be treated only after complete investigation, including the use of search methods. Otherwise, in every fifteenth case of a "red spot," a hidden carcinoma will be missed and treatment delayed. (Authors' abstract.)

CYTOLOGIC-COLPOSCOPIC OBSERVATIONS OF THE ECTOCERVIX OF PREGNANCY

WARREN R. LANG - Surgery, Gynecology, and Obstetrics 107: 31-36, 1958

Three hundred pregnant women were evaluated by cytology and colposcopy. One hundred ninety-eight were seen during various stages of normal pregnancy, 81 in the immediate postpartum period and 21 just before or after a spontaneous abortion. Frequent biopsies were taken.

The major effect of pregnancy on the cervix is that of hyperplasia. This is noticed with squamous and columnar epithelium by the appearance of papillomata and polyps respectively. Cervical erosion during pregnancy, as in non-pregnancy, usually consists of a perioral rim of columnar epithelium (ectopy) surrounded by a mixture of columnar and squamous epithelium (transformation zone). Cytologic atypias may arise in the latter area. Trichomonal infections may also be associated with an abnormal smear; colposcopically, leukoplakic areas may occur. Immediate postabortal and postpartal cervices show a minimal degree of laceration, bruising and loss of surface epithelium. The cytologic smear at this time contains erythrocytes, leukocytes, intermediate squamous cells and even cells of placental origin.

Colposcopic atypisms, without cancer, were noted in 22 instances. The smear was Class I in 19, Class II in 3 cases. Basal cell hyperactivity was found in 5 spot biopsies, all with negative smears. Two cases of invasive carcinoma (Stage I, Stage II) were also studied.

Cytology and colposcopy provide diverse yet complementary data concerning the uterine cervix of pregnancy. (Author's abstract.)

THE RELATIONSHIP OF CHRONIC VULVAR DISEASE, LEUKOPLAKIA, AND CARCINOMA IN SITU TO CARCINOMA OF THE VULVA

A. J. McADAMS, JR. and ROBERT W. KISTNER - Cancer 11: 740-757, 1958

There has been great confusion as to the significance of certain vulvar lesions in their relationship to the development of carcinoma. The majority of such lesions are likely manifestations of one or another of the various dermatitides. The special connotation attached to kraurosis vulvae cannot be supported and the term is to be avoided. If leukoplakia is defined on strictly histologic terms, using the same type of criteria for senile keratosis, a group of vulvar lesions of apparent significance can be separated. It is apparent, furthermore, that the majority of vulvar cancers are not unlike those appearing in the skin of other body locations. Of special interest in this study of vulvar cancer is the finding that when there are also changes of the type referred to as carcinoma in situ there is a more favorable prognosis. (Authors' abstract.)

THE NATURE OF THE CELLS ORIGINATING IN SO-CALLED "PRECANCEROUS" LESIONS OF THE UTERINE CERVIX

JAMES W. REAGAN - Obstetrical and Gynecological Survey 13: 157-177, 1958

The component cells represented in a comprehensive cellular sample provide detailed information about the parent tissue of origin. The cellular elements may indicate a normal epithelium of origin as well as the physiological status of the normal epithelium. Similarly, there may be evidence of abnormality. This may be manifested by the presence of a single cell type which is foreign to the normal cell population and is more or less characteristic of the reaction in the tissues. A decidual is demonstrated in this manner as is the presence of reserve cell hyperplasia and metaplasia. Other histopathological changes are more complex and have multiple properties and distinguishing features, many of which are evident by examining the component cells. As a result, in many instances it is readily possible to designate an abnormal reaction in the tissues by recognizing its basic characteristics as manifested in the cells. These basic features are not necessarily manifested in the same way in the tissues as they are in the cells.

There are certain properties which are more evident in cellular preparations than in conventional tissue studies, and conversely some histopathological features are apparently not present in the cellular preparations. Invasion per se is not evident on the basis of cellular evidence alone; however, it is possible in

some instances to distinguish between a precancerous change and a frank cancer by studying the component cells. This suggests that the surface change associated with frank cancer is not identical to the surface change observed in the so-called precancerous lesions, as has been suggested in the past. While the differences may be relative, there is nevertheless some basis for continuing the studies dealing with these respective cell types.

The cells desquamated from induced cancers in mice are significantly different from those observed in the normal estrous cycle and from those cells which originate in an altered surface change analogous to dysplasia in the human. Similarly, the cell forms which are observed in animals with induced cancer reflect the nature of the cancer. In previous studies a delicate linear cytoplasmic fibril was demonstrated in caudate and elongate forms arising in squamous cell cancers. Such a fibril might explain the configuration of the cell and also implies origin in a lesion that is characterized by abnormal keratin formation. These cell forms are commonly associated with keratinizing cancers in many different sites in both the human and the experimental animal.

While an abnormality in the surface mucosa may be evident in both histopathological and cytological preparations, at the present neither approach gives specific information as to the ultimate fate of a given lesion. By studying the desquamated cells it is possible to follow the course of a mucosal change without eradicating the parent lesion. Using such an approach, it has been demonstrated that some dysplastic lesions may undergo regression. Others persist for varying periods of time, and in some women a change interpreted as in situ cancer is ultimately found. Only rarely has invasive cancer been detected after prolonged surveillance. This approach is of value in following the course of dysplastic lesions demonstrated in pregnancy, since in many women the changes disappear or regress in the post partum period.

The basic properties of a surface change may be manifested in its cells. However, histopathological studies should be utilized in order to establish the exact nature of the lesion. As a pathologist with deep interest in the field of gynecological pathology, I am firmly convinced that cytology is a valuable adjunct in studying the uterine cervix. However, it is not a substitute for careful histopathological examination. In skilled hands, histology and cytology complement each other with the result that the overall accuracy of the examination is significantly increased when both procedures are employed.

The very comprehensive sampling of the cervical mucosa, which is afforded by a carefully obtained cellular specimen, may indicate the presence of an abnormality which is not evident in the histopathological sections prepared from the "biopsy" specimen. In such cases, deeper sections may be required in order to demonstrate the change while in other instances additional specimens must be obtained from the cervix. In recent years we have become aware of certain defects of our histopathological methods for studying the uterine cervix. Through the use of cytology some of these have come to our attention. More detailed histopathological studies can be made in cases in which the cellular evidence indicates a more serious alteration than is apparent in the initial tissue sections. On this evidence alone, it is possible to demonstrate small but out-spoken cancers which might otherwise have gone unrecognized. In addition, a detailed knowledge of cells and their structure gives the pathologist more insight in regard to the manifestations of disease in the tissue. (Author's summary.)

BEMERKENSWERTE, AUF "RADIO-MIMETISCHE" SCHAEDIGUNG DEUTENDE ZELLVERAENDERUNGEN BEI EINEM GRENZFALL ZUM INVASIVEN KOLLUMKARZINOM (CONSIDERABLE CELLULAR CHANGES INDICATING "RADIOMIMETIC" LESION IN A BORDERLINE CASE OF EARLY INVASIVE CARCINOMA)

I. H. MUELLER - Geburtshilfe und Frauenheilkunde 18: 471-476, 1958

A description is given of highly atypical cytologial alterations, suggestive of an energetic "radiomimetic" inhibition effect, observed in a borderline case of extremely early invasive carcinoma of the uterine cervix. The morphological evidence, resulting particularly from the findings of a separating oedematous zone, shows that no direct interaction occurs between the atypical epithelial cells and the surrounding connective tissue. This indicates a possible reactive cytotoxic factor, capable of limited diffusion. The potential nature of such a factor and the possibility of a viral aetiology of cervical cancer are discussed. (Author's summary.)

DIE EPITHELGRENZEN IM BEREICHE DES ISTHMUS UTERI (THE EPITHELIAL BORDERS IN THE AREA OF THE UTERINE ISTHMUS)

OBER, K. G., SCHNEPPENHEIM, P., HAMPERL, H., and KAUFMANN, C. - Archiv Fuer Gynaekologie 190: 346-383, 1958

The localization of the cervical mucous epithelium, endometrium and isthmus mucosa in relation to the internal os uteri was investigated in 120 uteri. The epithelial borders were defined by the PAS reaction. Histological sections including the whole organ were studied and localization of the anatomical internal os was estimated not only by microscopical criteria, but also histologically using Masson's connective tissue staining. In most instances, despite certain doubts in a few cases, both methods permitted a useful demarcation between corpus and cervical wall. Careful handling during removal of the uterus and

subsequent histological processing were carried out to minimize artefacts. The following results were obtained:

1. There was an isthmus mucosa between the endometrium and endocervical mucosa. The length of the isthmus mucosa varied considerably. It appeared to participate only slightly, if at all, in the cyclic changes of the endometrium.

2. The isthmus mucosa was not constantly localized in the same uterine segment. It was not possible in the histological section to recognize the isthmus segment by the structural characteristics of its wall.

3. Simultaneous application of macro- and microscopic criteria in the localization of the isthmus segment was impractical since it might give rise to false conclusions.

4. The isthmus mucosa in the case studied showed individual variation in localization around the internal os.

5. The distance from the highest to the lowest endocervical gland was remarkably constant in uteri of comparable sizes.

6. In uteri, where the lowest cervical gland was at the external os or even above, the isthmus mucosa was, as a rule, part of the corpus uteri.

7. The classical definition of isthmus uteri, as used by Aschoff, was true only if the lowest cervical gland was localized beyond the external os.

8. These findings showed that endocervical glands might extend to the pars vaginalis and for considerable distances, following deformities of the endocervix. The mechanism of this process was a smooth displacement of the soft inner against the stable outer layers of the cervix; one may regard this as a prolapse of the endocervical canal.

9. These findings were similar in nulliparous and multiparous women.

10. Only in a few cases was hyperplasia of cervical glands responsible for the evagination of the endocervical mucosa to the pars vaginalis.

11. This study led to an important clinical conclusion. As a rule, determination of the localization of the "last distal" cervical gland permitted a correct estimation of the localization of the histological internal os; therefore, the length of the cervical canal covered by mucous epithelium could be determined.

THE INDICATIONS FOR CONSERVATIVE THERAPY FOR INTRAEPITHELIAL CARCINOMA OF THE UTERINE CERVIX

JAMES S. KRIEGER and LAWRENCE J. McCORMACK - American Journal of Obstetrics and Gynecology 76: 312-320, 1958

The treatment of intraepithelial carcinoma of the uterine cervix can and should be individualized. Hysterectomy is not considered to be a therapeutic necessity. In the majority of cases radical conization with systematic cytologic follow-up has been found to be sufficient treatment. Hysterectomy is reserved for those patients in whom cervical spreads remain positive after conization, the cervix closes and renders spreads unreliable, or other major uterine disease is present. These conclusions as based on a series of 114 cases of carcinoma in situ of the cervix analyzed in regard to treatment and diagnostic findings. In 30 of these radical conization was followed in six weeks by total hysterectomy or its equivalent. 26 specimens were histologically negative for residual disease following hysterectomy. 65 patients were treated only by radical conization. One subsequently underwent hysterectomy, four have been lost to follow-up and 61 have been followed with cytologic techniques for from six months to seven years. (Authors' abstract.)

ZUR PROBLEMATIK DER FEINGEWEBLICHEN DIAGNOSTIK FRUEHER PLATTENEPITHELKARZINOMSTADIEN DES GEBAERMUTTERHALSES (PROBLEMS WITH HISTOLOGICAL DIAGNOSIS OF EARLY CERVICAL CARCINOMA)

K. H. BRUNTSCH - Geburtshilfe und Frauenheilkunde 18: 238-243, 1958

The paper presents a report concerning the case of a 28 year old patient in whom the diagnosis of carcinoma in situ of the uterine cervix had been made in February 1952. Without any therapeutic measures the lesion disappeared and normal epithelialization appeared, within a period of several months. Repeated cytological and colposcopical controls did not reveal any suspicious findings over a period of 5 years.

This case shows again that certain morphological pictures identified as "carcinoma in situ" do not deserve the terminology "carcinoma." A cooperative research project is recommended in which several

large institutes of Obstetrics and Gynecology would participate. In this study each case of so-called "carcinoma in situ" should be registered, and the histological sections of these lesions should be evaluated by the other histopathologists of the project. No therapy should be performed, but cytological and colposcopical controls should be made while the lesion is under observation.

After periods of 5 and 10 years, reports should be made before the Congress of the German Society of Gynecologists. Only by this means might it be possible to give a better evaluation of the lesion under discussion; however the author is aware that even this project will have some sources of error. (Author's abstract.)

KOLLUMKARZINOM MIT "CELLULES CLAIRES" (CARCINOMA OF THE CERVIX WITH "CELLULES CLAIRES")

H. ZEITZ and P. STOLL - Geburtshilfe und Frauenheilkunde 18: 753-760, 1958

Histological examination of 1260 cases of carcinoma of the cervix, grades I to IV, handled in this department from 1935 to 1951, brought to light 78 cases which were characterized by the predominance of "cellules claires" (Keller, Angel, Wittig). The development of this special type of carcinomatous structure is discussed and shown in several pictures. The prognosis of the cases is less favorable than that of the other types of squamous cell carcinoma of the cervix of the corresponding stages, but it is not entirely helpless. Radical surgery and/or radiotherapy resulted in a cure in 32.1% of these patients as compared with 48.2% in those cases without "clear cells" (stage I: 61.1% against 77.2%, stage II: 35.3% against 50.7%, stage III: 10% against 23.5% and stage IV: 0% against 1.5%). There was no appreciable difference in radio resistancy between the two types. (Authors' abstract.)

"CONSIDERACIONES ACERCA DE DOS CASOS DE SARCOMA ENDOMETRIAL (ESTROMATOSIS UTERINA)" (CONSIDERATIONS CONCERNING TWO CASES OF ENDOMETRIAL SARCOMA (UTERINE STROMATOSIS))

GRACIELA RAMIREZ A., EDUARDO VALDIVIA P., JORGE CAMPOS R. de C. - Ginecologia y Obstetricia 4: 90-102, 1958

Two cases of endometrial sarcoma have been reported, both of them with histological diagnoses after hysterectomy.

In the authors' opinion, this is a true sarcoma, of the lower malignant type, because of its capacity to infiltrate neighboring organs and to produce distant metastases.

The name used for this tumor has the advantage of precisely determining its malignant nature and the tissue where it takes origin. The endometrial sarcoma is different from the most common type of sarcoma of the uterus, the leiomyosarcoma of the myometrium. (Authors' abstract.)

ENDOMETRIAL SURFACE INVOLVEMENT BY CARCINOMA OF THE UTERINE CERVIX. REPORT OF TWO CASES

GILBERT H. FRIEDELL - Obstetrics and Gynecology 12: 179-184, 1958

In its more advanced stages epidermoid carcinoma of the cervix often extends upward for varying distances into the body of the uterus. It is rare, however, for the advancing neoplastic epithelium to remain superficial, that is, localized to the endometrial surface without penetration of the myometrium. It is even more unusual for the entire lining of the uterine cavity to be composed of malignant squamous epithelium.

Two cases of this type of carcinoma are reported in which the entire endocervical and endometrial surface was covered by apparent upward extension of neoplastic squamous epithelium from a primary cancer of the cervix. (Author's abstract.)

HORMONAL CYTOLOGY

LE FROTTIS VAGINAL A LA FIN DE LA GROSSESSE. NOTE PRELIMINAIRE. DEDUCTIONS PRATIQUES. (VAGINAL CYTOLOGY AT THE END OF PREGNANCY)

LICHTFUS, C., PUNDEL, J. P. and GANDAR, R. - Bull. Fed. Gynec. et Obstet. 9: 644-650, 1957

At the end of pregnancy, the vaginal smear presents important cytological changes, permitting one to see with an accuracy of over 90% if the pregnancy is not yet at term, is at term or has arrived at overmaturity. The three smear types (pregnancy before term, pregnancy at term and pregnancy with postpartum smear) are described. Artificial induction of labor, especially with continuous ocytocic perfusion, gives positive results only if the pregnancy smear is of the at-term or postpartum type. In the latter case, a single perfusion obtains nearly 100% immediate success.

This is a preliminary report which was presented at the Congress of Obstetrics and Gynecology of the French-speaking Societies held at Marseille in 1957. A detailed paper will be published in the near future.

During this Congress, other papers on vaginal cytology during pregnancy were presented:

Neumann, E., Fournie, G. and Gabriel, H.: Interet pratique des frottis vaginaux au cours de la gestation, Bull. Fed. Gynec. et Obstet. 9: 642-644, 1957.

Chosson, J., Serment, H. and Ruf, H.: Les frottis vaginaux en fin de grossesse, Bull. Fed. Gynec. et Obstet. 9: 650-652, 1957.

Both papers show the increasing interest of the obstetricians in vaginal cytology during pregnancy. The vaginal smears have now proved to be a simple, but very important and valuable, guide for the hormonal control of pregnancy and the diagnosis of the necessity to interrupt a pregnancy for fetal distress. (Authors' abstract.)

POST-PARTUM CERVICAL CYTOLOGY

RAYMOND H. KAUFMAN, NATHAN H. TOPEK and JACK P. ABBOTT. - Obstetrics and Gynecology 12: 189-194, 1958

Smears were obtained on 125 women on their initial post-partum visit (6-8 weeks). The initial smears were classified as follows: 85 (68%) class I, 25 (20%) class II, 14 (11.2%) class III, and 1 (.08%) class IV. The patients were divided into two groups: those who breast fed and those who bottle fed their babies. Of the 44 patient who breast fed, 11 (25%) were found to have class III smears. Of the 81 patients who did not breast feed, 3 (3.8%) had class III smears and one (1.2%) a class IV smear.

Of the 15 patients exhibiting class III or IV smears, 4 were lost to followup. One patient with a persistent class III smear over a 6 month period was found on cold knife cervical conization to have atypical epithelial hyperplasis of the cervix. In the remaining 10 patients the smears either reverted back to normal or multiple cervical biopsies did not reveal evidence or suspicion of malignancy.

The high percent of abnormal smears seen following delivery was believed related to the marked degree of cervical inflammation seen following childbirth as well as the cervical trauma during childbirth. The unusually high percent seen in the women who breast fed was felt to be related to a suppression of ovarian function with resultant thinning of the cervical epithelium. This possibly allowed the atypical basal cells, which are the result of infection and trauma incident to childbirth, to be shed more readily. (Authors' abstract.)

THE USE OF URINARY SEDIMENT AS AN AID IN ENDOCRINOLOGICAL DISORDERS IN THE FEMALE

CYRIL SOLOMON, PAUL PANAGEOTOPOULOS and ABRAHAM OPPENHEIM - American Journal of Obst. and Gyn. 76: 56-62, 1958

In the course of a study of urinary sediment for the diagnosis of tumor it has been found that epithelial cells in the urine were similar to those found in the vagina. It is believed that the female urethra responds in a way similar to the genital tract to endocrine stimulation. Since this is true, the study of urinary sediment reflects endocrinological disorders. It is therefore particularly recommended in young girls, in uterine hemorrhage and in vaginal infections which might interfere in the study of vaginal secretion. Routine smears can be made of urinary sediment in suspected miscarriages and abortions to study the percentage of keratinized and non-keratinized cells. By this method a better prognosis may be obtained. (Authors' abstract.)

DIE BEZIEHUNGEN DES SCHLEIMEPITHELS ZUM PLATTENEPITHEL AN DER CERVIX UTERI IM LEBENSLAUF DER FRAU (THE RELATIONSHIP OF COLUMNAR AND SQUAMOUS EPITHELIUM OF THE UTERINE CERVIX DURING THE LIFE SPAN OF THE WOMAN)

SCHNEPPENHEIM, P., HAMPERL, H., KAUFMANN, C., and OBER, K. G. - Archiv fuer Gynaekologie 190: 303-345, 1958

This report is based on the study of 697 uteri and 156 cervices of women of all ages, including surgical specimens as well as autopsy material. They were investigated with regard to the localization of squamo-columnar junction of the cervical epithelium. Histological sections of the whole organ were studied. Removal of the uterus and subsequent histological processing were carried out with utmost care in order to minimize the occurrence of artefacts. This report does not include the study of carcinoma in situ and early stages of invasive carcinoma. Preoperative colpophotography and gross photography before and after fixation were done on all surgical specimens to prevent any misinterpretation. Results of this study showed the following:

1. The squamo-columnar junction was very rarely located exactly at the external os. This localization was found in one case only.

2. The relationship of these epithelia to each other showed great variations and appeared to be related to age and ovarian function.

3. Before puberty the squamous epithelium usually extended as far as the external os, often also reaching into the cervical canal. Cervical glands were almost always confined to the cervical canal.

4. During the reproductive years the cervical glands often extended to the portio vaginalis. There the endocervical glands either communicated freely with the surface or were overgrown by squamous epithelium.

5. After the menopause the cervical glands were restricted to the endocervical canal. In elderly women their localization were usually clearly defined above the external os. In these women the squamous epithelium extended more or less into the endocervical canal. In some instances it even reached as far as the internal os.

6. During the menopause the histology of the endocervical canal was indistinguishable from that of the pars vaginalis in most women of childbearing age.

7. After 30 years of age the squamous epithelium tended to grow into the endocervical canal (as shown in plate 6, Ie, IIa and IIe) irrespective of the localization of the lowest cervical gland.

8. The histological reports of the investigator's material agreed well with the colpophotographical findings. The discrepancy in 25 per cent of the cases may have two explanations:

   (a) Erroneous interpretation of endocervical epithelium as an area of erosion due to eversion of the pars vaginalis during colposcopy.

   (b) Especially in older women, what is diagnosed by colposcopy as transformation zone with cysts, may be enlarged cervical glands which are displaced under the epithelium of the ectocervix overgrown by squamous epithelium with their openings in the endocervix.

9. Whereas in younger women the relation of squamous and columnar epithelium was characterized by an erosion of the columnar epithelium, inflammation and insufficient epidermization, in older women the usual finding consisted of more pronounced epidermization.

10. During pregnancy erosions of the columnar epithelium with insufficient epidermization were frequently found in addition to the physiological changes occurring at the squamo-columnar junction. (Authors' summary.)

THE CYCLICAL CHANGES IN THE MUCOSA OF THE GUINEA PIG'S UTERUS, CERVIX AND VAGINA AND IN THE SEXUAL SKIN, INVESTIGATED BY THE ELECTRON MICROSCOPE

MARIO H. BURGOS and GEORGE B. WISLOCKI - Endocrinology 63: 106-121, 1958

The mucosa of the guinea pig's endometrium, cervix and vagina and the epithelium of the perivaginal (sexual) skin have been studied with the electron microscope during estrus, metestrus and late diestrus.

During estrus, the uterine epithelial cells, the superficial mucosa cells of the cervical epithelium, as well as the basal and prickle cells of the vaginal epithelium display microville on their surfaces; these disappear during diestrus.

Throughout the cycle in the cervix and during diestrus in the vagina, the superficial epithelial cells are filled with large droplets of a low-density material, apparently mucus. This seems to arise within distended vesicles of the endoplasmic reticulum in lower cells.

During estrus in the vagina and at all times in the sexual skin, the uppermost epithelial cells consist mainly of filaments. In the basal cells, these filaments occur in small clusters anchored on thickenings on the cells surfaces. Such cells contain an abundance of dense cytoplasmic particulates presumed to be ribonucleoprotein; and endoplasmic reticulum is absent or poorly developed.

The transitional layer of the vaginal epithelium during estrus shows a gradual retraction and apparent dissolution of the nuclei, paralleled by a swelling vacuolization and disappearance of the mitochondria.

Some correlations have been drawn between the present observations and those reported earlier (Endocrinology 59: 93, 1956) for the histochemical characteristics of these same tissues. (Authors' summary.)

LACTATION PERIOD IN INDIAN WOMEN - DURATION OF AMENORRHEA AND VAGINAL AND CERVICAL CYTOLOGY

HANNAH PETERS, SARAH ISRAEL, and SAVITRI PURSHOTTAM - Fertility and Sterility 9: 134-144, 1958

1. The duration of lactation amenorrhea was studied in 272 Indian women. The frequency of the recurrence of menses was determined in the different postpartum months. The usual lactation amenorrhea was found to be much longer in Indian women than that reported in women from the United States and Europe.

2. Vaginal smears were taken on women in the postpartum period. These were compared with smears taken from the exocervix at the same time. The tabulation of the different smear types indicated that the cervical mucosa reacts less sensitively to hormone stimulation than the vaginal epithelium. As the endometrial epithelium responds less sensitively to sexual hormones than the vagina, the response of the cervical epithelium, as revealed in the cervical smear, can be used to give a closer insight into the functional state of the endometrium in the lactation period. (Authors' summary.)

EXFOLIATIVE CYTOLOGY OF THE SENILE FEMALE URETHRA

V. H. YOUNDBLOOD, E. M. TOMLIN, J. O. WILLIAMS, and P. KIMMELSTIEL - Journal of Urology 79: 110-113, 1958

Exfoliative cells were obtained from the urethra by means of a small non-absorbent cotton tipped applicator. The slides were fixed and stained by the routine method as described by Papanicolaou. The smears were taken from postmenopausal patients whose ages vary from 45 to 102 years (average 61 years).

The predominant cells, "basal, parabasal, intermediate, or superficial," were reported. These studies were done in conjunction with a clinical study to determine the effectiveness of suppositories in treating senile urethritis. Smears done prior to treatment revealed a predominance of basal and parabasal cells indicative of atrophic change. These patients were then treated with urethral suppositories containing furacin and 0.1 mg. of diethylstilbesterol. In most cases, after two weeks treatment with these suppositories, the exfoliative cells were observed to be intermediate or superficial type squamous epithelial elements. This exfoliative cytology study indicates that atrophic epithelial elements in the urethra may be converted to superficial squamous epithelial cells simply by the local application of estrogens. (Authors' abstract.)

CYTOLOGICAL TECHNIQUES

STAINING REACTIONS OF BASIGRANULAR AND CARCINOID TUMOR CELLS; WITH SPECIAL REFERENCE TO A MODIFIED GIEMSA METHOD

JACK KEVORKIAN - The American Journal of Clinical Pathology 30: 37-44, 1958

A study of carcinoid tumors is presented with the results of special staining procedures done in an attempt to find fairly specific granular color reactions. Granules failed to stain with the following procedures: periodic acid-Schiff, methyl green-pyronin, thionin, Gomori iron reaction, and the Warthin silver impregnation. Hazy staining was noted with the azan, pyronin Y, and Gomori chromhematoxylin-phosphomolybdic acid technics. Unna's methylene blue and Masson trichome stains revealed distinct granules only if they were numerous. Granules were not acid-fast. Satisfactory results were obtained with slightly acidified Wright's stain; but a modified Giemsa method, which imparted a bright distinct orange-brown to the granules against a background of light blue-green nuclei and fainter, almost colorless cytoplasm, gave the best results and may be of value in the diagnosis of questionable examples of carcinoid tumors. (Author's abstract.)

CERVICAL CELL STUDIES -- A METHOD OF INCREASING PRODUCTION

WILLIAM M. CHRISTOPHERSON and JAMES E. PARKER - The Journal of the American Medical Association 166: 1718-1719, 1958

More than 24,000 slides of cervical cell studies from 12,000 women were examined. The material was collected by means of a cervical swab using non-absorbent cotton applicators. In all cases, duplicate slides were examined. Sixty-one of these patients with abnormal cell studies subsequently had the diagnosis of carcinoma of the cervix made by tissue examination. In sixty of the sixty-one cases both slides showed abnormal cells. The first slide prepared almost always contained the best material for adequate examination. In a single case the first slide contained abnormal cells and the second preparation contained insufficient cells and was considered unsatisfactory. It was felt that by obtaining and examining a single slide the specificity of cervical cell studies obtained in this manner would not be greatly altered. Thus the productivity of a cytology unit could be perhaps doubled by eliminating examination of multiple slides from a given patient. (Authors' abstract.)

TETRAZOLIUM STAINING IN CYTOLOGY OF THE UTERINE CERVIX

PETER M. MARCUSE - Obstetrics and Gynecology 11: 707-714, 1958

Cervical scrapings were stained with solutions of neotetrazolium. Positive reactions were characterized by deposits of colored particles representing the formazan that was formed after reduction of the tetrazolium. This cytochemical method was compared with the conventional procedures based on histology and exfoliative cytology. The principle of supravital staining with tetrazolium was also applied to the intact cervix of surgically removed uteri where it resulted in a grossly visible coloration of the mucosa. A positive reaction was not a specific expression of succinic dehydrogenase activity, as shown by various modifications of the basic technic. The series comprised preparations from epidermoid cervical carcinomas, from benign lesions and from normal cervices. A preponderance of formazan deposits was noted in the preparations derived from carcinomas. This association of positive results with malignant lesions was not ascribed to any special quality of the cancer cells but was explained by the distribution of cells capable of reducing the tetrazolium. Normally these cells are confined to the basal layer of the epithelium while in cases of carcinoma they are present throughout the entire epithelial thickness. (Author's abstract.)

SMEAR-SECTION CORRELATION: A CYTOLOGIC TEACHING AID

ALVAN G. FORAKER & GLADYS C. CLARK - American Journal of Clinical Pathology 30: 45-46, 1958

Since medical students and physicians are more familiar with hematoxylin and eosin stained sections than cytologic preparations, standard sections and Papanicolaou stained impression smears of the same normal and tumorous tissues (and cells) have been prepared on the same slide. This permits quick microscopic comparison of the pertinent cells prepared by both methods and, in practice at the University of Florida, has facilitated teaching of the cytologic method to students. (Author's abstract.)

VERMEIDBARE TECHNISCHE FEHLER DEI DER HERSTELLUNG ZYTOLOGISCHER PRÄPARATE- (THE POSSIBILITY OF AVOIDING TECHNICAL ERRORS IN THE PREPARATION OF CYTOLOGICAL SLIDES)

HANS-JÜRGEN SOOST - Zentralblatt fur Gynakologie 80: 622-627, 1958

Discussed here are some of the changes which arise through technical errors in the preparation of cytological slides and the avoidance of these mistakes. In particular, the following are considered: (1) well known changes from drying before fixation, (2) staining disturbances due to insufficient fixation, (3) appearance of hematoxylin sedimentation, (4) the deposition of talcum and starch grains, (5) the observation of special granules which arise in stained slides before covering them with Canada Balsam. (Author's abstract.)

UEBER DEN EINFLUSS DER IN DER KOLPOSKOPIE ANGEWANDTEN REAGENZIEN AUF DAS ZELLBILD DES PORTIOABSTRICHES (THE INFLUENCE OF THE REAGENT SOLUTIONS USED IN COLPOSCOPY ON THE CYTOLOGIC PATTERN OF THE CERVICAL SMEAR)

BERISLAV BERIE and HORST SMOLKA - Geburtshilfe und Frauenheilkunde 18: 852-864, 1958

For the early diagnosis of carcinoma of the cervix the cytologic smear technique is today often combined with colposcopy, with the object of achieving the greatest possible diagnostic accuracy. This combination is useful only if the two methods do not interfere with each other, i.e., that the steps involved in taking vaginal and cervical smears do not disturb or distort the appearance of the cervix in such a way as to render colposcopy unreliable, and vice versa. Investigations were carried out with the view of ascertaining whether, and to what extent, the two methods interfere with each other. Special reference was given to the influence of the reagent solutions used in colposcopy on the cytologic smear. The results were discussed and suggestions were made as to how the two techniques can be combined to the best advantage. (Authors' abstract.)

OTHER PHASES OF CYTOLOGY

A CYTOLOGICAL STUDY OF LYMPH NODE IMPRINTS

JOHN E. ULTMANN, IRENA KOPROWSKA, and RALPH L. ENGLE, Jr. - Cancer 11: 507-524, 1958

The structure of normal and diseased lymph nodes was studied from imprints to determine the diagnostic value of the imprints. The cut surface of freshly excised, bisected lymph nodes was touched lightly to slides that were then either air dried and stained by the Wright-Giemsa technique or fixed with ether-alcohol and stained with Papanicolaou EA-65 stain.

Ninety-three lymph nodes were diagnosed as showing benign disease and 73 as showing malignant disease. Chronic lymphadenitis was characterized by variability of lymphoid cells, ranging from any small to fewer medium and large lymphocytes. Reticulum cells, plasma cells, histiocytes, and tissue mast cells were also seen. Subacute lymphadenitis differed from chronic lymphadenitis only the presence of granulocytes. In tuberculosis, epitheloid and Langhans type giant cells were seen. Primary malignant tumors of lymph nodes were characterized by the relative uniformity of the cell type. In the various lymphosarcomas, predominance of small lymphocytes, lymphoblasts, or reticulum cells was of diagnostic importance. In Hodgkin's disease, variability of cell type was the rule; however, the findings of Reed-Sternberg cells with or without eosinophilia made the diagnosis possible. Metastatic cells in lymph nodes were recognized with ease by their size, staining variability, large nuclei, bizarre nucleoli, frequent cluster arrangement and occasional vacuolated cytoplasm. Consistency by correlation between the routine pathological section studies and the two imprint method was good. (Authors' summary.)

DEHYDROGENASE ACTIVITY OF ENDOMETRIUM - APPLICATION OF TETRAZOLIUM STAINING METHODS TO SMEARS AND SECTIONS FROM SURGICAL SPECIMENS

PETER M. MARCUSE - The American Jounal of Clinical Pathology 28: 539-548, 1957

Impression smears from the endometrium of surgically removed uteri were incubated in solutions of tetrazolium and were examined for intracytoplasmic deposits of crystals of formazan. This smear method proved to be suitable for the cytologic study of the reduction of tetrazolium. For this specific purpose, impression smears provided certain advantages over the use of frozen sections. A set of smears was made from each specimen of endometrium, and the individual preparations were treated with variations of the basic method. After completion of the procedure, the smears were graded according to the amount of the deposits of formazan and 4 ratings were estimated for each of the sets. These values reflected the degree to which the reduction of the tetrazolium was affected by freezing and by the presence of succinate. The results indicate that the activity of the succinic dehydrogenase in the endometrium is greater in the proliferative stage than during the secretory stage. Evaluation of the individual ratings further suggests that the activity of dehydrogenase in any specimen of endometrium is characterized by the relative distribution of succinic dehydrogenase, nonspecific reductase and a nonenzymatic type of reduction. (Author's summary.)

THE EFFECT OF CROTON OIL AND CARCINOGENS ON MITOSIS AND THE DEOXYRIBONUCLEIC ACID CONTENT OF CERVICAL EPITHELIAL CELLS

D. G. SCARPELLI and EMMERICH von HAAM - Cancer Research 18: 657-663, 1958

Microspectrophotometric analysis of nuclear DNA and mitotic counts revealed that a correlation exists between increased nuclear DNA and augmented mitoses, or mitotic rate. Endocervical application of croton oil or 20-methylcholanthrene resulted in augmented mitoses and increased exfoliation of epithelial cells containing greater amounts of nuclear DNA. Distinct differences appear to exist between the mitogenic effect of croton oil and methylcholanthrene on the genital epithelium of the mouse. Marked increases in the mitotic rate and nuclear DNA (polyploidy) were observed in many malignant cells. (Author's abstract.)

ÜBER DEN ZELLIGEN BESTAND DES STROMA DER MENSCHLICHEN CORPUSMUKOSA (ABOUT THE CELLULAR COMPOSITION OF THE STROMA OF THE HUMAN UTERINE MUCOSA)

F. FEYRTER - Archiv für Gynäkologie 190: 47-82, 1957

1. The "fixed" cells of the syncytial reticulocellular stroma of the human uterine mucosa appear in varying forms, as far as the size of the cells, the size of the nuclei and the nuclear chromatin are concerned.

2. Different "free" cells (the histiocytes and histiocytic round cells, the monocytic round cells and the lymphocytic round cells) come out of the reticulocellular syncytium - which represents the mesenchymal cell stem in this locality. These show marked tendency to phagocytize and to store, especially with the purpose of removing cellular debris and decomposed material.

3. The lymphocytic and histiocytic endometrial "Körnerzellen" (V. Numers, Literature; Hamperl) are a very remarkable form of the storing round cells. The "Körner" (granula) are amphophile (oxyphil and chromotrop), azurophil and contain acid mucopolysaccharids (Asplund and Holmgren) bound to proteins (Hellweg, Hellweg and Sandritter). Their cytoplasm frequently appears to be somewhat colorless and edematous. The granular storage apparently occurs in indirect dependence to the effect of ovarian hormones on the intercellular ground substances of the uterine stroma; in the latter, the metabolism of mucoproteid plays a special role, to which perhaps not enough attention is paid. As far as the nuclei of "Körner" cells are concerned, there is a remarkable tendency for hyperploidy and occurrence of so-called amitotic figures during nuclear division. Heavy functional stress leads to peculiar polymorphy and peculiar karyorrhexis.

4. In addition to examination of slides of fixed tissue during the study of cells of the uterine stroma, the staining of imprints, especially with Pappenheim's method, was used with success. (Author's abstract.)

THE NEXT SYMPOSIUM

VOLUME III 1959 NUMBER 1

The Symposium by Correspondence of the next edition is devoted to the discussion of:

CYTOLOGY DURING PREGNANCY

The subjects for discussion cover normal and disturbed pregnancy and cervical carcinoma during pregnancy.

Speakers are listed alphabetically.

Symposium A

CANCER CYTOLOGY DURING PREGNANCY

1. The Squamo-Columnar Junctions during Pregnancy

   KARL-GÜNTHER OBER, Cologne, Germany

   Disc.: Alvan G. Foraker, Jacksonville, Florida, U.S.A.
   Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
   Louis J. Zeldis and Dean L. Moyer, Los Angeles, California, U.S.A.

2. Incidence of Cervical Carcinoma during Pregnancy

   JORGE CAMPOS R. de C., Lima, Peru
   JOHN B. GRAHAM, Buffalo, New York, U.S.A.
   THOMAS A. SLATE, San Diego, California, U.S.A.
   PETER STOLL, Heidelberg, Germany

   Disc.: George J. Andros, Philadelphia, Pennsylvania, U.S.A.
   Ronald R. Greene, Chicago, Illinois, U.S.A.
   Emmerich von Haam, Columbus, Ohio, U.S.A.
   Edmund Schüller, Vienna, Austria

3. Prognosis of Cervical Carcinoma during Pregnancy as Compared with the Prognosis of Cervical Carcinoma in Non-Pregnant Women

   WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
   GENEVIEVE DALIAN, A. SIMATOS and VIOLETTE M. NUOVO, Paris, France
   HERBERT E. NIEBURGS, New York, New York, U.S.A.
   EDMUND SCHÜLLER, Vienna, Austria

   Disc.: Alfred Glücksmann, Cambridge, England, U.K.
   Jules-André Bret, Paris, France
   Peter Stoll, Heidelberg, Germany

4. Histomorphology of Cervical Carcinoma during Pregnancy

   FRIEDRICH BAJARDI, Graz, Austria
   JEAN A. de BRUX, Paris, France
   JORGE CAMPOS R. de C., Lima, Peru
   ROGER VOKAER, Brussels, Belgium

Disc.: Alvan G. Foraker, Jacksonville, Florida, U.S.A.
Alfred Glücksmann, Cambridge, England, U.K.
Emmerich von Haam, Columbus, Ohio, U.S.A.
Herbert E. Nieburgs, New York, New York, U.S.A.
Peter Stoll, Heidelberg, Germany
Louis J. Zeldis and Dean L. Moyer, Los Angeles, California, U.S.A.

5. Histomorphology of Cervical Decidual Reaction

JEAN A. de BRUX and JACQUELINE DUPRÉ-FROMENT, Paris, France

6. Colposcopy during Pregnancy and the Post-Partum Period (on Normal and Abnormal Cervices)

JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France
WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.

Disc.: Friedrich Bajardi, Graz, Austria
Robert Ganse and Raimund Krimmenau, Dresden, Germany
Otakar Nyklíček, Náchod, Czechoslovakia
Hans Iselin Wyss, Zurich, Switzerland

7. Colpomicroscopy on Normal and Abnormal Cervices during Pregnancy

TASSILO ANTOINE, KURT BRANDL, VIKTOR GRÜNBERGER, E. KOFLER and HANNES KREMER, Vienna, Austria
WOLFGANG WALZ, Heidenheim a.d., Brenz, Germany

8. Exfoliative Cytology of Infections during Pregnancy

EMMERICH von HAAM, Columbus, Ohio, U.S.A.
HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc.: Luis Montalvo-Ruiz, Madrid, Spain

9. Exfoliative Cytology of Dysplasia during Pregnancy

GENEVIEVE DALIAN, A. SIMATOS and VIOLETTE M. NUOVO, Paris, France
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
ROBERT E. L. NESBITT, Jr., DORIS S. ROME and ARTHUR A. STEIN, Albany, New York, U.S.A.
GUILLERMO TERZANO, Buenos Aires, Argentina

Disc.: George J. Andros, Philadelphia, Pennsylvania, U.S.A.
Jean A. de Brux, Paris, France
Camille Lichtfus, Athus, Belgium
Dean L. Moyer and Louis J. Zeldis, Los Angeles, California, U.S.A.

10. Exfoliative Cytology of Carcinoma in Situ during Pregnancy

JEAN A. de BRUX and JACQUELINE DUPRÉ-FROMENT, Paris, France
GENEVIEVE DALIAN, A. SIMATOS and VIOLETTE M. NUOVO, Paris, France
HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc.: Anthony F. Anderson, Edinburgh, Scotland, U.K.
George J. Andros, Philadelphia, Pennsylvania, U.S.A.
J. Ernest Ayre, Miami, Florida, U.S.A.
Hanns-Werner Boschann, West-Berlin, Germany
Jacques W. Jenny and Alfred Wacek, Zurich, Switzerland

11. Exfoliative Cytology of Invasive Carcinoma during Pregnancy

EMMERICH von HAAM, Columbus, Ohio, U.S.A.

Disc.: Hanns-Werner Boschann, West-Berlin, Germany
Jorge Campos R. de C., Lima, Peru

12. Clinical Viewpoints with Special Consideration to Therapy and Time of Therapy for Lesions of the Uterine Cervix during Pregnancy

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France

Disc.: George J. Andros, Philadelphia, Pennsylvania, U.S.A.
Hanns-Werner Boschann, West-Berlin, Germany
Edmund Schüller, Vienna, Austria

13. Diagnostic Accuracy of Colposcopy as Compared to Cytology in the Detection of Cervical Carcinoma during Pregnancy

FRIEDRICH BAJARDI, Graz, Austria
JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France

Disc.: Alfonso Alvarez-Bravo, Mexico, D.F., Mexico
Clarice do Amaral Ferreira, Rio de Janeiro, Brazil
Jacques W. Jenny and Hans Iselin Wyss, Zurich, Switzerland
Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
Hans Muth, Münster, Germany

14. Should All Pregnant Women be Screened for Cervical Carcinoma?

EMMERICH von HAAM, Columbus, Ohio, U.S.A.
LEOPOLD G. KOSS, New York, New York, U.S.A.
THOMAS A. SLATE, San Diego, California, U.S.A.

Disc.: George J. Andros, Philadelphia, Pennsylvania, U.S.A.
J. Ernest Ayre, Miami, Florida, U.S.A.
Jules-André Bret, Paris, France
Ronald R. Greene, Chicago, Illinois, U.S.A.
Jacques W. Jenny, Zurich, Switzerland
Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
Violette M. Nuovo, Paris, France
Yoseup S. Song, Providence, Rhode Island, U.S.A.

15. Exfoliative Cytology and Colposcopy of Cervical Decidual Reaction

JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France
ELIE TASSO, ANDRÉ RAUZY and JEAN A. de BRUX, Paris, France

Disc.: Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
Violette M. Nuovo, Paris, France

Symposium B

HORMONAL CYTOLOGY DURING PREGNANCY AND POST-PARTUM PERIOD

1. The Rate of Production of Endogenous Hormones in Pregnancy

JOSEF ZANDER, Cologne, Germany

Disc.: Ernst Jürgen Plotz, Chicago, Illinois, U.S.A.

2. Hormonal Basis of Cytological Changes during Pathological Pregnancy

OTTO STAMM and GUSTAVE RIOTTON, Geneva, Switzerland

3. Epithelial and Connective Tissue during Pregnancy

JEAN A. de BRUX, Paris, France

4. Normal Vaginal Cytology during Pregnancy

EMMERICH von HAAM and THOMAS D. EFSTATION, Columbus, Ohio, U.S.A.
BERTHOLD B. HOCHSTAEDT, Haifa, Israel
HERBERT E. NIEBURGS, New York, New York, U.S.A.
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Marcel Gaudefroy, Lille, France
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Raimund Krimmenau, Dresden, Germany
José Maria E. Mezzadra and Guillermo Terzano, Buenos Aires, Argentina
Hans Muth, Münster, Germany

5. Incidence of Cytolysis in Smears during Pregnancy

MARCEL GAUDEFROY, Lille, France
EMMERICH von HAAM and ROBERT SCHWALLENBERG, Columbus, Ohio, U.S.A.
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Camille Lichtfus, Athus, Belgium
Luis Montalvo-Ruiz, Madrid, Spain
Herbert E. Nieburgs, New York, New York, U.S.A.

6. Vaginal Flora of Pregnant Women as Compared to That of Non-Pregnant Women

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
JULES-ANDRÉ BRET, Paris, France

Disc.: Emmerich von Haam, Columbus, Ohio, U.S.A.
Berthold B. Hochstaedt, Haifa, Israel
J. Paul Pundel, Luxembourg, Luxembourg

7. Vaginal Cytology as Prognostic Method in Pregnancy Disorders

MARCEL GAUDEFROY, Lille, France
LUIS MONTALVO-RUIZ, Madrid, Spain
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Jules-André Bret, Paris, France
Jacques Ferin, Louvain, Belgium
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
José Maria E. Mezzadra and Guillermo Terzano, Buenos Aires, Argentina
Herbert E. Nieburgs, New York, New York, U.S.A.
Erica Wachtel, London, England, U.K.

8. Effect of Administered Estrogens on the Vaginal Epithelium during Pregnancy and the Post-Partum Period

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Jacques Ferin, Louvain, Belgium
Marcel Gaudefroy, Lille, France
František Horálek and Mojmír Sonek, Brno, Czechoslovakia
Luis Montalvo-Ruiz, Madrid, Spain

9. Vaginal Cytology at the End of Pregnancy

CAMILLE LICHTFUS, Athus, Belgium
NILO P. LUZ, Porto Alegre, Brazil
LUIS MONTALVO-RUIZ, Madrid, Spain
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: František Horálek and Mojmír Sonek, Brno, Czechoslovakia
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Otakar Nyklíček, Náchod, Czechoslovakia
Gustave Riotton, Geneva, Switzerland
Guillermo Terzano and José Maria E. Mezzadra, Buenos Aires, Argentina

10. Vaginal Cytology after Rupture of Fetal Membranes

B. CORNELIS HOPMAN, Miami, Florida, U.S.A.

Disc.: Emmerich von Haam, Columbus, Ohio, U.S.A.
František Horálek and Mojmír Sonek, Brno, Czechoslovakia
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Violette M. Nuovo, Paris, France
J. Paul Pundel, Luxembourg, Luxembourg

11. Vaginal Cytology Post-Partum and During the Lactation Period

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil
WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.
OTAKAR NYKLÍČEK, Náchod, Czechoslovakia
HANNAH PETERS, Bombay, India
ROGER VOKAER, Brussels, Belgium

Disc.: František Horálek and Mojmír Sonek, Brno, Czechoslovakia
Herbert E. Nieburgs, New York, New York, U.S.A.
Violette M. Nuovo, Paris, France
J. Paul Pundel, Luxembourg, Luxembourg
Yoseup S. Song, Providence, Rhode Island, U.S.A.
Guillermo Terzano and José Maria E. Mezzadra, Buenos Aires, Argentina

12. Vaginal Cytology in Abortion

GIUSEPPE DELLEPIANE, Torino, Italy
CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil
OTTO STAMM, V. RAWYLER and GUSTAVE RIOTTON, Geneva, Switzerland

Disc.: Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Herbert E. Nieburgs, New York, New York, U.S.A.

13. Vaginal Cytology in Ectopic Pregnancy

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina

Disc.: J. Ernest Ayre, Miami, Florida, U.S.A.
Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Jean A. de Brux, Paris, France

14. Diagnosis of Pregnancy by Means of Cytology

B. CORNELIS HOPMAN, Miami, Florida, U.S.A.

Disc.: Marcel Gaudefroy, Lille, France
František Horálek and Mojmír Sonek, Brno, Czechoslovakia
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
J. Paul Pundel, Luxembourg, Luxembourg

15. Urinary Cytology During Pregnancy

MARIO GONZÁLES-RAMOS, Mexico, D.F., Mexico
GUILLERMO TERZANO and ARTURO A. ARRIGHI, Buenos Aires, Argentina

16. Correlative Studies on Vaginal, Urethral, Oral Smears and Smears from the Urinary Sediment During Normal Pregnancy

PIERO SORA, Pavia, Italy

Disc.: Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany

FUTURE SYMPOSIA

VOLUME III 1959 NUMBER 2

The symposia by correspondence of Volume III, Number 2, 1959, will be devoted to the discussion of:

RADIATION CELL CHANGES

DEFINITION, MORPHOLOGY, CYTOCHEMISTRY, DIAGNOSTIC AND PROGNOSTIC IMPORTANCE OF RADIATION CHANGES OF BENIGN (RR–) CELLS AND OF TUMOR CELLS IN THE FEMALE GENITAL TRACT

1. **Definition**
2. **Morphology**
3. **Cytochemistry of Irradiated Cells**
4. **Phasemicroscopy**
5. **UV-Microscopy**
6. **Fluorescence Microscopy**
7. **Colpomicroscopy of Irradiated Cervix**
8. **Colposcopy of Irradiated Cervix**
9. **Tissue Culture**
10. **Animal Experiments**
11. **Minimal r-Dosages of X-ray or Minimal mg-E-h Dosages of Radium Which Produce Radiation Cell Changes**
12. **Histological Criteria of Radiation Response**
13. **Prognosis by Means of Histology (Method)**
    (a) **Prognosis based on tumor type and on presence of lymphatic emboli**
    (b) **Prognosis based on serial biopsies**
14. **Do Serial Biopsies Disturb the Healing Process of the Irradiated Cervical Carcinoma?**
15. **Prognosis by Means of Exfoliative Cytology (Method)**
16. **Comparative Studies of Exfoliative Cytology and Histology After Irradiation**
17. **Does Irradiation Influence the Karyopyknotic Index?**
18. **Recurrent Carcinoma and Presence of Radiation Cell Changes**
19. **Is There a Difference in Cellular Response (Concerning Onset of Response, Quality and Quantity) if Irradiation Has Been Only by X-Ray, Only by Radium, or Both? If so, What are the Differences?**
20. **Is There a Difference in Cellular Response, (Concerning Onset of Response, Quality and Quantity) if Irradiation Has Been Only by X-ray, Radiation and Cobalt?**

21. Are the Degree and the Duration of Radiation Cell Changes Dependent on the Dosage of Irradiation or the Period of Time Over Which It is Given?

22. Can the Cytological or Histological Response to Irradiation be Influenced by Medication or Other Factors?

23. Clinical Viewpoint: Should a Lesion be Treated with Surgery After it Has Been Shown Cytologically That There is Not Any – or Only Slight – Radiation Response Present? If so, When Should Surgery be Performed?

24. Clinical Viewpoint: Radical Surgery in Cases of Recurrent Cervical Carcinoma After Irradiation

25. Which Clinical Factors are Associated with a Good Cytological Response?

26. Cytological Response as Compared with Local Primary Healing and with Local Recurrence and Metastases of Cervical Carcinoma

27. Comparison of Radiation Cell Changes in Exfoliated Vaginal Cells and Exfoliated Cells Elsewhere, e.g., Oral Carcinoma

28. How Soon After the Beginning of Therapy Can the Radiation Response be Judged Accurately?

29. Results of RR-Cell Studies (Minimal Requirements for Participants in this Topic: 100 Patients Studied Over a Period of 5 Years)

30. Cytological Studies on Chemical Protection of the Rectum During Pelvic Irradiation

PLEASE NOTE: THE SECOND SYMPOSIUM PLANNED FOR THIS EDITION ENTITLED

SYMPOSIUM ON SR-CELLS

WILL BE HELD AT A LATER DATE.

VOLUME III 1959 NUMBER 3

The Symposia by correspondence of Volume III, No. 3, 1959, will be devoted to the discussion of two main subjects:

A. EFFECTS OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM
B. VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR CYTOLOGICAL STUDIES

Deadlines for Contributors to the Symposia of this Edition:

FOR RECEIPT OF MAIN PAPERS:

Main papers in the English language must REACH ACTA CYTOLOGICA, Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: July 1, 1959.

Main papers in French, German or Spanish must REACH ACTA CYTOLOGICA, Editorial Office NOT LATER THAN: June 10, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF DISCUSSION:

THE DISCUSSANTS WILL RECEIVE THE PAPERS OF THE MAIN SPEAKERS FOR COMMENTS AS SOON AS THEY ARE AVAILABLE.

Discussions in the English language must REACH ACTA CYTOLOGICA, Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: September 15, 1959.

Discussions in French, German or Spanish must REACH ACTA CYTOLOGICA, Editorial Office, NOT LATER THAN: August 20, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF CLOSING REMARKS BY MAIN SPEAKERS:

THE MAIN SPEAKERS WILL RECEIVE THE DISCUSSIONS FOR CLOSING REMARKS AS SOON AS THEY ARE AVAILABLE.

Closing remarks in the English language must REACH the Editorial Office NOT LATER THAN: December 1, 1959. Closing remarks in French, German or Spanish must REACH the Editorial Office NOT LATER THAN: November 10, 1959.

PROGRAM OF VOLUME III, NUMBER 3, 1959

Symposium A

EFFECTS OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM

1. Introduction. Sources of Endogenous Estrogens
2. Histological Changes in the Epithelium and Connective Tissue of the Vagina and Ectocervix as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation
3. Cytological Changes of Vaginal and Ectocervical Epithelium as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation
4. Cytological Changes of the Endocervical Epithelium as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation
5. Cytochemistry of Exfoliated Atrophic Cells (Basal-Parabasal Cells)
6. Cytochemistry of Exfoliated Intermediate Cells
7. Cytochemistry of Exfoliated Superficial Cells and Anucleated Squamous Cells
8. Cytochemistry of Cytoplasmic Granules
9. Terminology of Cytological Smears in Regard to Estrogen Effect
10. Is the Karyopyknotic Index a Measurement of Endogenous Estrogen?
11. Is the Presence of Epithelial Atrophy a Definite Criteria of Lack of Estrogen Production?
12. Cellular Degeneration (Cytolysis, Autolysis) and Endogenous Estrogens
13. The Value of Exfoliative Cytology in the Diagnosis of Ovulation
14. The Value of Exfoliative Cytology in the Diagnosis of Follicular Persistency
15. Vaginal Cytology in Hysterectomized Patients, with Special Reference to Presence or Absence of Ovarian Function After Hysterectomy
16. The Value of Exfoliative Cytology in the Diagnosis of Hormone-Producing Tumors
17. The Level of Endogenous Estrogens in Patients with Cervical Carcinoma as Demonstrated by Methods Other than Exfoliative Cytology
18. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Cervical Carcinoma
19. The Level of Endogenous Estrogens in Patients with Ovarian or Endometrial Carcinoma as Demonstrated by Methods Other Than Exfoliative Cytology
20. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Ovarian or Endometrial Carcinoma
21. The Level of Endogenous Estrogens in Patients with Breast Carcinoma as Demonstrated by Methods Other than Exfoliative Cytology
22. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Untreated, Treated and Recurrent Breast Carcinoma (Excluding Those Cases Receiving Hormone Therapy or Who Have Undergone Extirpation of Endocrine Organs)
23. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Breast Carcinoma after Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroids Administrated-Considered Separately)
24. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Breast Carcinoma after Adrenalectomy With or Without Additional Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroids Administrated-Considered Separately)
25. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Breast Carcinoma After Hypophysectomy With or Without Additional Adrenalectomy and/or Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroids Administered-Considered Separately)
26. Ovarian Function Following Pelvic Irradiation As Assessed by Vaginal Smears
27. The Vaginal Smear During Menopause

Symposium B

ADVANTAGES AND DISADVANTAGES OF VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR ROUTINE CYTOLOGICAL EXAMINATIONS

1. Review of Techniques of Vaginal Smears
2. Review of Techniques of Cervical Smears
3. Review of Techniques of Endocervical Smears
4. Review of Techniques of Intra-uterine Smears
5. Material Obtained by Pipette from Vaginal Pool Only, With Visualization of Cervix
6. Material Obtained by Pipette from Vaginal Pool Only, After Insertion of Speculum
7. Material Obtained by Vaginal Tampon Only
8. Material Obtained by Cervical Scraping Only
9. Material Obtained by Two Techniques: (a) Vaginal Smears (b) Cervical Smears
10. Material Obtained by Two Techniques: (a) Cervical Smears (b) Endocervical Smears
11. Material Obtained by Three Techniques: (a) Vaginal Smears (b) Cervical Smears (c) Endocervical Smears
12. Material Obtained by Sponge-Biopsy
13. If One Prepares More Than One Smear Per Patient, Could One Prepare These Smears on One Glass Slide or Does One Have to Put Them on Separate Glass Slides?
14. Material Obtained by Use of the Blade of a Bivalve Speculum, Smearing with Gloved Finger
15. In a Case of a Suspicious Ectocervix, Should a Biopsy Be Done Without Cytology?
16. A Comparison of Blind Aspiration of the Vaginal Fluid and Aspiration Under Visualization

VOLUME IV 1960 NUMBER 1

The Symposium by Correspondence of this edition will be devoted to the discussion of:

CARCINOMA IN SITU AND SO-CALLED PRECANCEROUS LESIONS

Deadlines for Contributors to the Symposia of this Edition:

FOR BEING LISTED AS SPEAKER OR DISCUSSANT:

In order to be listed as Main Speaker or Discussant on any of the topics listed in the following program, participants should inform the Editorial Office as soon as possible; however, NOT LATER THAN: July 1, 1959, about their intention to contribute.

FOR RECEIPT OF MAIN PAPERS:

Main papers in the English language must REACH ACTA CYTOLOGICA, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: September 1, 1959.

Main papers in French, German or Spanish must REACH ACTA CYTOLOGICA NOT LATER THAN: July 20, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF DISCUSSIONS:

THE DISCUSSANTS WILL RECEIVE THE PAPERS OF THE MAIN SPEAKERS FOR COMMENTS AS SOON AS THEY ARE AVAILABLE.

Discussions in the English language must REACH ACTA CYTOLOGICA, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: November 15, 1959.

Discussions in French, German or Spanish must REACH ACTA CYTOLOGICA NOT LATER THAN: October 15, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF CLOSING REMARKS BY MAIN SPEAKERS:

THE MAIN SPEAKERS WILL RECEIVE THE DISCUSSIONS FOR CLOSING REMARKS AS SOON AS THEY ARE AVAILABLE.

Closing remarks in the English language must REACH ACTA CYTOLOGICA NOT LATER THAN: February 1, 1960. Closing remarks in French, German or Spanish must REACH the Editorial Office NOT LATER THAN: January 1, 1960.

PRELIMINARY LIST OF TOPICS OF SYMPOSIA OF VOLUME IV, 1960, NUMBER 1

CARCINOMA IN SITU AND SO-CALLED PRECANCEROUS LESIONS

Introduction

SPECIAL PANEL DISCUSSION ON HISTOLOGICAL TERMINOLOGY (a specially invited panel will discuss the definitions of the following conditions):

Normal Epithelium
Ectopy and Ectropion
Epidermization and Atypical Epidermization
Leukoplakia and Parakeratosis
Reserve Cell Hyperplasia
Basal Cell Hyperplasia
Abnormal Epithelium (School of Zurich)
Undifferentiated Regenerative Epithelium
Dysplasia
Carcinoma in Situ
Early Invasive Carcinoma (Microcarcinoma)
Invasive Carcinoma

The discussion of the above definitions will be based on photomicrographs and histomorphological descriptions.

Individual Topics

Symposium A

PROBABLE OR POSSIBLE PRE-MALIGNANT CERVICAL LESIONS

Cervicitis and Endocervicitis

1. Histomorphology of Cervicitis and Endocervicitis
2. Histochemistry of Cervicitis and Endocervicitis
3. Exfoliative Cytology of Cervicitis and Endocervicitis
4. Cytochemistry of Cervicitis and Endocervicitis
5. Colposcopy of Cervicitis and Endocervicitis
6. Colpomicroscopy of Cervicitis and Endocervicitis
7. Clinical Viewpoint: Management of Cervicitis and Endocervicitis
8. Interrelationship: Cervicitis-Endocervicitis and Cervical Carcinoma

Ectopy, Ectropion and Epidermization

9. Histomorphology of Ectopy, Ectropion and Epidermization
10. Histochemistry of Ectopy, Ectropion and Epidermization
11. Exfoliative Cytology of Ectopy, Ectropion and Epidermization
12. Cytochemistry of Ectopy, Ectropion and Epidermization

13. Colposcopy of Ectopy, Ectropion and Epidermization
14. Colpomicroscopy of Ectopy, Ectropion and Epidermization
15. Clinical Viewpoint: Management of Ectopy, Ectropion and Epidermization
16. Interrelationship: Ectopy-Ectropion-Epidermization and Cervical Carcinoma

Leukoplakia

17. Histomorphology of Leukoplakia
18. Histochemistry of Leukoplakia
19. Exfoliative Cytology of Leukoplakia
20. Cytochemistry of Leukoplakia
21. Special Microscopic Studies on Leukoplakia (Interference-, UV-, Electron-, Phase-, or Fluorescence-Microscopy)
22. Animal Experiments
23. Colposcopy of Leukoplakia
24. Colpomicroscopy of Leukoplakia
25. Clinical Viewpoint: Management of Leukoplakia
26. Interrelationship: Leukoplakia and Cervical Carcinoma

Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

27. Histomorphology of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
28. Histochemistry of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
29. Exfoliative Cytology of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
30. Cytochemistry of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
31. Special Microscopic Studies on Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia (Interference-, Electron-, Phase-, UV-, or Fluorescence-Microscopy)
32. Animal Experiments
33. Colposcopy of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
34. Colpomicroscopy of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
35. Clinical Viewpoint: Management of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
36. Interrelationship: Reserve Cell Hyperplasia-Basal Cell Hyperplasia-Dysplasia and Cervical Carcinoma

Symposium B

PROBABLE OR POSSIBLE MALIGNANT CERVICAL LESIONS: CARCINOMA IN SITU

Histology of Carcinoma in Situ

1. Histomorphology of Carcinoma in Situ
2. What Constitutes a Definite Diagnosis of Carcinoma in Situ with Special Reference to Amount of Histological Sections Required ("Serial Sections") and Type of Material?
3. What is Not Carcinoma in Situ?
4. What Percentage of Cervices Show Early Invasion in Serial Histological Sections in Uteri Which Were Removed Under the Biopsy Diagnosis of "Carcinoma in Situ"?
5. Nomenclature of the Atypical Epithelium
6. The Atypical Non-Invasive Zones Around Invasive Carcinoma (Cervical)
7. Histochemistry of Carcinoma in Situ
8. Special Microscopic Studies on Histological Sections of Carcinoma in Situ (Interference-, Electron-, UV-, Fluorescence-, or Phasemicroscopy)

Inspection Techniques on Carcinoma in Situ

9. Colposcopy of Carcinoma in Situ
10. Colpomicroscopy of Carcinoma in Situ

11. Schiller Test in Carcinoma in Situ

Exfoliative and Experimental Cytology of Carcinoma in Situ

12. Cytomorphology of Carcinoma in Situ
13. Cytochemistry of Carcinoma in Situ
14. Phasemicroscopy on Exfoliated Cells of Carcinoma in Situ
15. Ultraviolet Microscopy on Exfoliated Cells of Carcinoma in Situ
16. Fluorescent Microscopy on Exfoliated Cells of Carcinoma in Situ
17. Electron Microscopy on Exfoliated Cells of Carcinoma in Situ
18. Interference Microscopy on Exfoliated Cells of Carcinoma in Situ
19. Can One Differentiate Carcinoma in Situ from Invasive Carcinoma by Means of Exfoliative Cytology?
20. Carcinoma in Situ of the Uterine Cervix as the Primary Cytological Diagnosis
21. Exfoliative Cytology Following Irradiation of Carcinoma in Situ
22. Experimental Carcinoma in Situ (Animal Experiments)
23. Tissue Culture of Cells from Carcinoma in Situ

Clinical Viewpoint on Carcinoma in Situ

24. Advantages and Disadvantages of Conservative Management of Carcinoma in Situ (with special reference to possible recurrence, fatal outcome and postoperative pregnancy)
25. Advantages and Disadvantages of Radical Surgical Management of Carcinoma in Situ
26. Advantages and Disadvantages of Irradiative Management of Carcinoma in Situ
27. Management of Recurrent Carcinoma in Situ after Cone Biopsy
28. Advantages or Disadvantages for the Clinical Management of Cytologic Prediction of the Extent and Nature of a Cervical Lesion (Invasive or Non-Invasive) if this Prediction were Correct in at Least 80% of the Cases
29. May one Treat the "Early Invasive Carcinoma (Microcarcinoma)" Less Radically that the More Extensive Invasive Carcinoma?

Morphogenesis and Incidence of Carcinoma in Situ

30. Incidence of Carcinoma in Situ with Special Reference to Age, Pregnancy, Parity, Race and Hormonal Condition
31. Probable Percentage of Invasive Carcinoma Passing Through the Stage of Carcinoma in Situ
32. Latent Period of Carcinoma in Situ
33. Probable Percentage of Regression of Carcinoma in Situ
34. Do Surgical Procedures on Cervices with Carcinoma in Situ Increase the Incidence of Postoperative Invasions?
35. Cervical Carcinoma in Situ as Compared with Carcinoma in Situ in Other Sites.

SYMPOSIA UNDER CONSIDERATION

The following symposia have been suggested for consideration and are not listed in the order of preference or chronology. You are invited to inform the Editorial Office in which of the symposia you would be most interested, so that an order of preference may be tentatively arranged.

1. Symposium on Tadpole-Shaped Squamoid Cells.
2. Symposium on Organization of Laboratory of Exfoliative Cytology.
3. Symposium on Cytological Studies in Amenorrhea.
4. Symposium on Cytology of Ascitic Fluid.
5. Symposium on Cytology of Malignant Tumors of Ovary and Tubes.
6. Symposium on Extra-Genital Cytology of Metastatic Gynecological Lesions.
7. Symposium on Phasemicroscopy and Other Special Microscopic Techniques.
8. Symposium on Training of Exfoliative Cytologists.
9. Symposium on Cytological Changes due to Microbiological Factors.
10. Symposium on the Comparative Diagnostic Accuracy, Efficiency and Specificity of Techniques for Detection of Carcinoma.
11. Symposium on Histiocytes.
12. Symposium on Cytological Microphotography.
13. Symposium on Cytological Terminology for Hormonal Evaluation.
14. Symposium on Quantitative Cytochemistry of Exfoliated Cells.
15. Symposium on Sex Chromatin.
16. Symposium on Vaginal Cytology During Childhood.
17. Symposium on Lung Cytology.
18. Symposium on Cytology of Exudates.
19. Symposium on Gastrointestinal Cytology.
20. Symposium on Endocervical Adenocarcinoma.
21. Symposium on Training of Cytotechnicians.
22. Symposium on Effect of Progestational Agents on the Vaginal Epithelium.
23. Symposium on Oral Cytology.
24. Symposium on Cytology of Nipple Discharge.
25. Symposium on Squamous Cell Cancer of the Cervix with Particular Emphasis on Micro-invasive Lesions.
26. Symposium on Incidence Statistics of Cervical Carcinoma.
27. Symposium on Tumor Cells in the Peripheral Blood.
28. Symposium on Terminology such as "Abnormal", "Suspicious", "Doubtful" and Others.
29. Symposium on the Menopause.

WANTED OR AVAILABLE

It is the purpose of this column to promote international exchange of cytologists and cytotechnicians, to inform them of open permanent positions, and to inform employers of available cytology personnel. Persons interested in obtaining permanent positions as cytologists or cytotechnicians or in obtaining temporary fellowships in cytology (teaching, exchange, or training fellowships), and individuals or institutions offering such positions or openings are invited to write giving full information to: ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A. Information supplied will be held strictly confidential.

While information received is subject to editing so that it conforms to the style of ACTA CYTOLOGICA, ACTA CYTOLOGICA cannot and do not assume responsibility for statements made by contributors.

OFFRES ET DEMANDES

Cette rubrique est destinée à favoriser l'échange international de cytologistes et de techniciens en cytologie. Elle renseignera sur les places permanentes vacantes et informera également sur le personnel cytologique disponible. Les personnes désirant obtenir une place permanente de cytologiste ou cytotechnicien, ou faire un stage temporaire en cytologie (enseignement, échange, training), et les instituts ou personnes offrant de telles places sont invités à écrire aux ACTA CYTOLOGICA (5841, Maryland Avenue, Chicago 37, Illinois, U.S.A.) en donnant tous les détails. Les informations reçues auront un caractère strictement confidentiel.

Les annonces reçues devront être, pour la publication, rédigées dans le style des ACTA CYTOLOGICA, mais les ACTA CYTOLOGICA ne peuvent accepter aucune responsabilité pour l'exactitude des renseignements fournis par les annonceurs.

STELLENANGEBOTE UND STELLENGESUCHE

Mit dieser Rubrik soll internationaler Stellenaustausch und Stellenvermittlung für Zytologen und zytologisch-technische Assistenten angebahnt werden, indem über offene Stellungen und über verfügbares Personal berichtet wird. Zytologen und zytologisch-technische Assistenten und Assistentinnen, die an vorübergehenden (Lehrstellen, Austauschstellen, Lernstellen) oder dauernden Anstellungen interessiert sind, und Personen oder Institutionen, die derartige Stellunge zu vergeben haben, sind gebeten an ACTA CYTOLOGICA (5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.) zu schreiben und möglichst genaue Einzelheiten anzugeben. Die erhaltenen Auskünfte und Einzelheiten werden streng vertraulich behandelt.

ACTA CYTOLOGICA kann keine Verantwortung für Angaben übernehmen, die von Beitragenden zu dieser Rubrik gemacht werden.

SOLICITUDES Y OFERTAS

El propósito de esta sección es promover el intercambio internacional de citólogos y técnicos en Citología, informar de vacantes en puestos permanentes, y de personal citológico disponible. Las personas que estén interesadas en obtener becas temporales, o puestos permanentes como citólogos o técnicos en Citología (Enseñanza, Intercambio, Becas de aprendizaje), y, asimismo, las personas o instituciones que puedan ofrecer tales puestos, deben escribir a ACTA CYTOLOGICA (5841 Maryland Avenue, Chicago 37, Illinois, USA) aportando información completa. Esta información será estrictamente confidencial.

Cuando las informaciones recibidas sean para su publicación en ACTA CYTOLOGICA, la Revista, no puede asumir, ni asume, la responsabilidad de los informes o afirmaciones hechas por los contribuyentes.

CYTOLOGISTS AND CYTOTECHNICIANS WANTED

CYTOLOGIST IN CHARGE WANTED for University Department of Obstetrics and Gynecology. Candidates must be Doctor of Medicine interested in teaching, research and special studies in Exfoliative Cytology. Foreign candidates must govern the English language, and be acceptable to the local Medical State Board. The appointment would be initiated with the rank of Instructor with an annual salary of $7200.00, subject to merit increases in salary and promotion in rank. Candidates are requested to submit their curriculum vitae with bibliography (including available reprints of publications) and three references (out of whom at least one must be from the United States) to Russel R. de Alvarez, M.D., Professor and Executive Officer, Department of Obstetrics and Gynecology, University of Washington, Seattle 5, Washington, U.S.A.

CHIEF CYTOTECHNICIAN WANTED for the Cytology Laboratory of the Cancer Cytology Survey Program of Rhode Island. This senior cytology checker will be given a supervising duty as well as an opportunity to teach cytology students. The salary is $4862 per annum. The work week is 35 hours with two weeks and three days vacation per year.

Write for details to: Dr. Y. S. Song, Director in Charge (Pathologist) for Cancer Cytology Survey of Rhode Island, 593 Eddy Street, Providence 2, Rhode Island.

MEDICAL RESEARCH TECHNOLOGIST WANTED trained or willing to be trained as a cytotechnician for work in the Department of Obstetrics and Gynecology at L.S.U. School of Medicine, Louisiana State Civil Service Employee; starting salary $3500.

QUALIFIED CYTOTECHNICIAN WANTED full-time for Department of Pathology of Medical College of Georgia, Augusta. Opportunities for research and teaching in medical school environment. New Laboratories, well-equiped and air-conditioned. Attractive salary.

Apply: Dr. L. D. Stoddard, Professor of Pathology, Medical College of Georgia, Augusta, Georgia.

RESEARCH FELLOW IN GYNECOLOGIC PATHOLOGY WANTED. The Baptist Memorial Hospital, Jacksonville, Florida, will offer a Research Fellowship in the general area of research in gynecologic pathology to begin in the summer of 1959. The hospital currently operates 272 beds. It was opened in September, 1955, in a new air-conditioned building directly on the St. Johns River. The laboratories are attractively located on the second floor with separate laboratories for Histology, Bacteriology, Hematology, Clinical Microscopy and Chemistry. A new research laboratory with animal facilities is located on the sixth floor. The hospital is approved for residency training in Pathologic Anatomy and Clinical Pathology. Requirements: Graduation from an approved medical school, one year of approved internship, and at least one year of approved residency training in pathology or in obstetrics-gynecology. Stipend: A stipend of $3800 per year with an additional $350 per year for one or two dependents. No maintenance is furnished. Uniforms are not furnished but are laundered without charge.

Applications and inquiries should be addressed to: Alvan G. Foraker, M.D., Pathologist, Baptist Memorial Hospital, 800 Miami Road, Jacksonville 7, Florida.

RESIDENCY FOR FOREIGN GRADUATE (Other than French) in Obstetrics and Gynecology offered in the Maternite de l'Hopital de Creteil, Paris, France. The applicant should have experience in surgical obstetrics and gynecology (caesarean sections, forceps, hysterectomy) prior to application. The applicant will receive training in general obstetrics and gynecology and will have the opportunity of working in the laboratories, especially in the cytology laboratory of Professor Jean de Brux. The opening for this position will be either November, 1959 or April, 1960, and is for the period of one year. The monthly salary is 45.000 to 50.000 French francs. For further details write to Docteur A. J. Bret, Chef de Service de la Maternite de l'Hopital de Creteil, 53 Avenue de Saxe, Paris 7, France.

CYTOLOGISTS AND CYTOTECHNICIANS AVAILABLE

CYTOLOGICALLY TRAINED RESIDENT AVAILABLE for appointment in University Hospital in Germany or in the United States of America. Applicant (citizen of West-Germany, male) was trained for one year in a teaching laboratory in the United States approved by the American Board of Pathology for training of pathologists. Applicant has finished a complete internship in the United States and is available for an appointment as of January 1, 1960. Please write to Code "MD-1-1959" to ACTA CYTOLOGICA, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOLOGIST-GYNECOLOGIST, Female, Age: 34, citizen of West Germany, single, graduate of West German Medical School (M. D. - Dr. Med.)

Medical Training:	Eligible for German Board of Obstetrics and Gynecology (Facharzt fuer Frauenheilkunde). Completed additional internship and full residency training in Obstetrics and Gynecology in the United States of America.
Cytology Training:	(1) in West German Medical School, Department of Obstetrics and Gynecology and (2) one and a half years full time Cytology Fellowship in a training center in the United States (approved by the American Cancer Society).
Wanted:	Position as gynecologist in charge of Cytology Laboratory (with clinical work if desirable) in The United States of America, Canada or West-Berlin, Germany.
Code No.:	OMD 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOLOGIST-PATHOLOGIST, Male, Age: 36, single, graduate from Italian University (M.D.), citizen of Italy.

Medical Training:	Pathologist with six years experience after completion of residency.
Cytology Training:	Self-trained.
Wanted:	Training Fellowship in Cytology or Research Associate in Cytology in Austria, Germany or Switzerland for 6 to 12 months.
Code No.:	LLM 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

GYNECOLOGIST, Male, Age: 33, citizen of Denmark, married, graduate of Danish University (M.D.).

Medical Training:	Completed internship and residency in Obstetrics and Gynecology, and 3-1/4 years staff member in University Hospital.
Cytology Training:	None.
Wanted:	Training Fellowship in Cytology in Great Britain or Central Europe.
Code No.:	ANV 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 25, single, citizen of the United States of America, University Graduate.

Cytology Experience:	Presently Chief-Cytotechnician in cytology Laboratory in a Medical School in the United States of America. This particular laboratory is a training laboratory approved by the American Cancer Society, co-author of several scientific publications on cytology.
Wanted:	Teaching Fellowship for 3-4 months to organize, set-up, or modernize cytology laboratories. Would consider India, Australia, New Zealand, Ireland or Africa. Will return to present position in United States upon completion of fellowship.
Code No.:	UG 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 32, citizen of West Germany, single, registered medical technician.

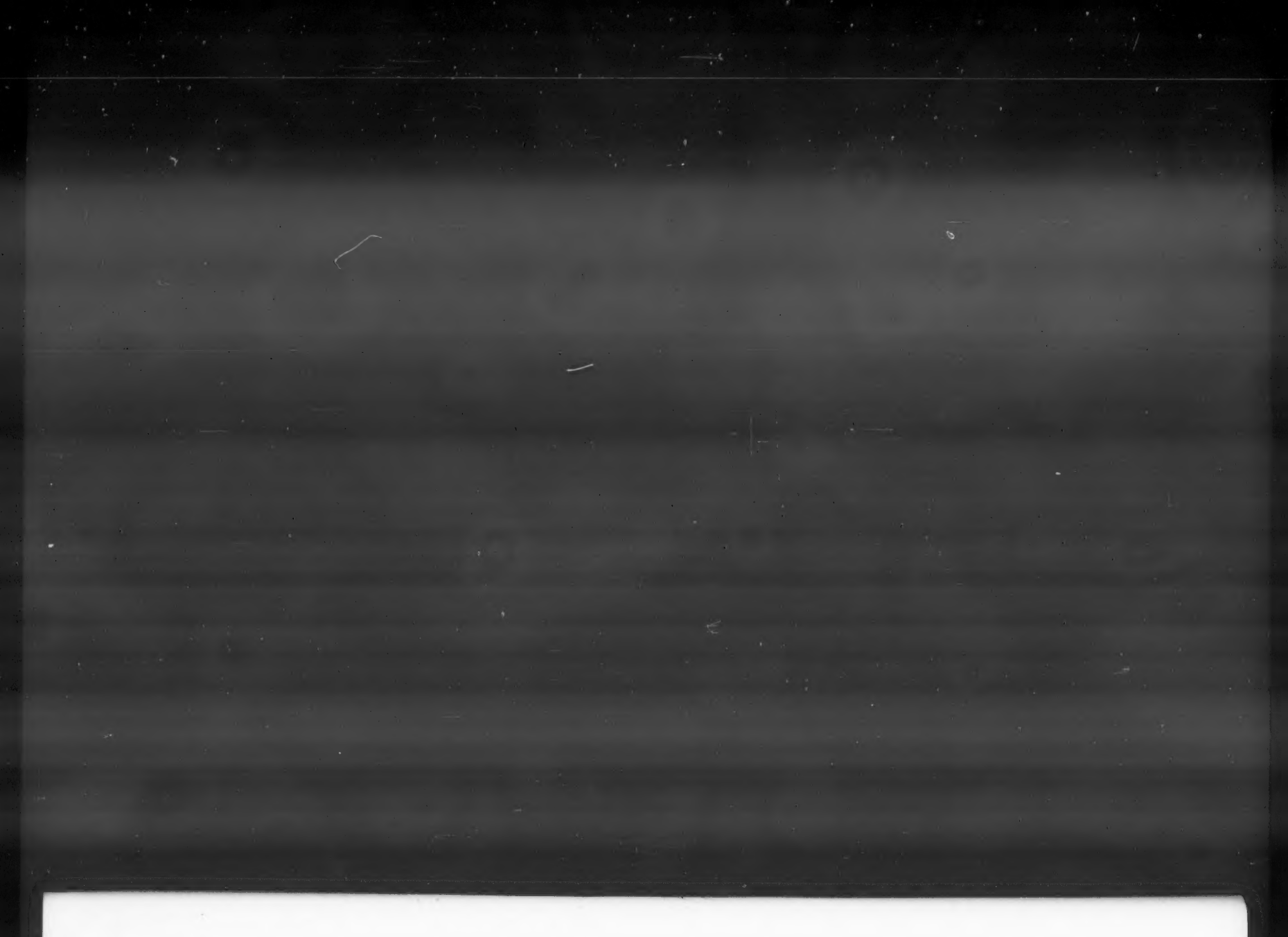
Cytology Experience:	Chief-Cytotechnician 7 years in cytology laboratory of a University Department of Obstetrics and Gynecology in Germany. Experience in cancer cytology, endocrinological cytology and hematology.
Wanted:	Exchange fellowship for a period of several months with a cytology center in the United States of America, Brazil or Argentina. Will return to present position upon completion of fellowship.
Code No.:	RU 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

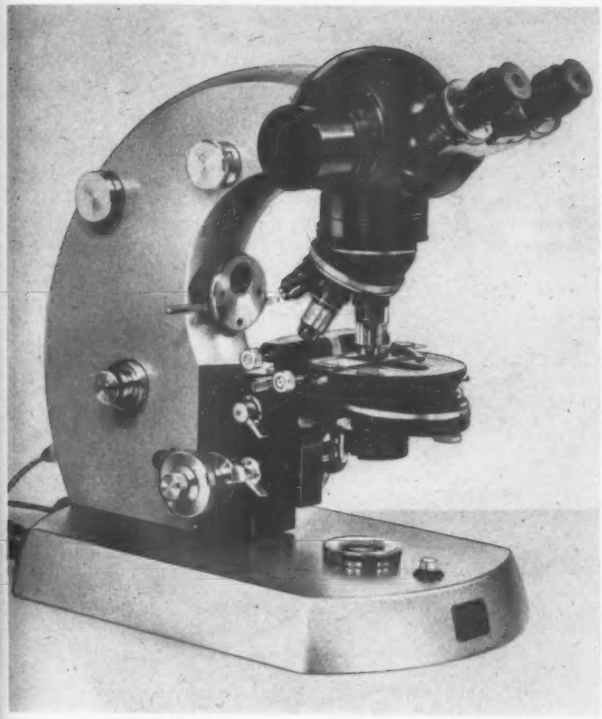
TRAINED CYTOTECHNICIANS AVAILABLE FOR SCREENING: Two cytological technicians trained in approved teaching laboratory, experienced in cancer screening and cytohormonal evaluations of cell smears, are available to process and screen material on a part-time basis for physicians. Specimens may be sent by mail and will be reported within a few days. Charges are made per specimen. Interested pathologists and physicians are asked to write to: Code HOME-SCREENING to ACTA CYTOLOGICA, 5841 S. Maryland Avenue, Chicago 37, Illinois, U.S.A.

COURSES IN EXFOLIATIVE CYTOLOGY

COURS DE TECHNICIENNES DE CYTOLOGIE CLINIQUE sous le patronage de l'Assistance de la Prévention Générale de la Sécurite Sociale et de la Faculté de Médecine de Paris. Un cours de techniciennes de Cytologie aura lieu du 25 Mai au 6 Juin 1959. Ce cours est réservé aux laborantines déjà rompues aux techniques microscopiques, Le nombre d'élèves est strictement limité à 15. Les candidates devront fournir avec leur demande d'inscription un curriculum vitae. Les Cours et le Travaux Pratiques auront lieu l'après-midi de 15 a 19 heures. La première semaine sera consacrée à l'enseignement théorique et aux démonstrations adaptées. La seconde semaine à la coloration et à la lecture des lames. Division du Cours: 1. Physiologie et Histologie Normale, 2. Morphologie Cellulaire, 3. Prélèvements et Colorations, 4. Exfoliation cyclique, puberté, menopause, grossesse, 5. Inflammation et infestations, 6. Lésions inflammatoires et dysplasiques du col, 7. Cancer du col, 8. Dysplasies et Cancer Intra-épithélial, 9. Cancer du corps utérin et Liquides d'épanchement, 10. Cancers broncho-pulmonaires, 11. Recherche des élements anormaux dans les urines.

S'inscrire auprès du Professeur agrégé Jean de Brux, Laboratoire de Colpocytologie, Hopital Broca, 111, rue L. M. Nordmann, Paris 13, France. Un certificat d'assiduité sera délivré à la fin du Cours. Participation aux frais: 5000 francs.





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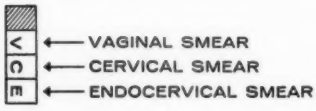

Sketches to illustrate the preparation of the cytological smears on the Glasco "VCE" slide: (1) an ordinary wood spatula is used to obtain the specimen (without scraping) from the mucosa of the lateral wall of the vagina, and then the material is spread on the portion of the slide marked "V" adjacent to the frosted end; (2) a second wood spatula is used to scrape the mucosa of the uterine cervix (especially any recognizable abnormal area), and this material is spread on the middle section marked "C"; (3) a cotton-swab applicator (preferably premoistened) is inserted into the endocervix and rotated slightly, and then the material is spread on the portion of the slide marked "E".

Wied, G. L.—Amer. J. Clin. Path. 25: 742-750, 1955
Wied, G. L.—Amer. J. Clin. Path. 28: 233-242, 1957

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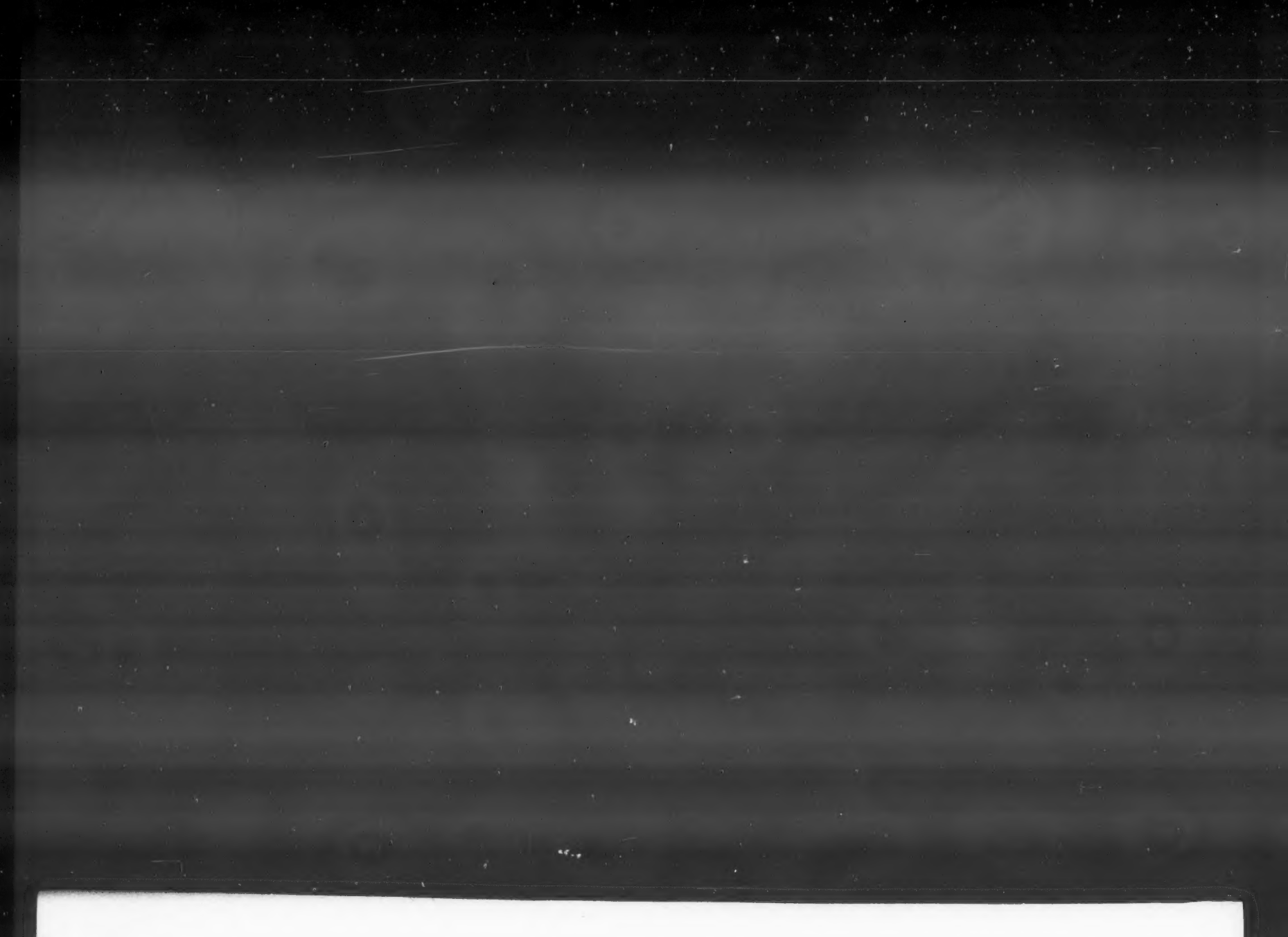
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